ARCHIVES OF PATHOLOGY

VOLUME 30 SEPTEMBER 1940 NUMBER 3

ETIOLOGY OF ACUTE HEMORRHAGIC PANCREATITIS WITH SPECIAL REFERENCE TO THE VASCULAR FACTORS

AN ANALYSIS OF AUTOPSIES AND AN EXPERIMENTAL INVESTIGATION

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The genesis of acute hemorrhagic pancreatitis is still unknown, although it has been the subject of repeated clinical and experimental study. Since 1855, when Claude Bernard[1] first attempted to induce this disease in dogs by injecting bile mixed with sweet oil into the pancreatic duct, many methods have been used to produce it. So far, however, the mechanism of development has not been demonstrated in a manner free from criticism. The events transpiring during the earlier stages, although of utmost importance to a complete understanding of the disease, are the least well understood aspects of it. Most writers agree that the problem of the genesis of acute pancreatic necrosis is yet unsolved.

This communication attempts to evaluate the importance of circulatory disturbances as basic factors in the mechanism of the production of acute hemorrhagic pancreatitis. The more acceptable current theories of its causation are reviewed with special reference to those dealing with vascular changes. The circulatory changes observed in 40 cases are summarized, and the experimental production of acute pancreatic necrosis in 21 dogs by intra-arterial injection of droplets of metallic mercury is reported.

THEORIES OF ORIGIN

Common Channel Theory.—The most frequently accepted explanation for the production of acute hemorrhagic pancreatitis is that relating to a reflux of bile into the pancreatic duct. This hypothesis was first suggested by Lancereaux,[2]

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1. Bernard, C.: Leçons de physiologie expérimentale, appliquée à la médecine, Paris, J. B. Baillière, 1855, vol. 2, p. 278.

2. Lancereaux, E.: Traité des maladies du foie et du pancréas, Paris, O. Doin, 1899; cited by Mann and Giordano.[6]

in 1899. The possibility of this accident depends on the anatomic arrangement of the orifices of the pancreatic and common bile ducts. Opie,[3] in 1901, described a case of acute pancreatitis in which a gallstone was lodged in the ampulla of Vater. Blockage of the common outlet of these two ducts, he said, converted them into one continuous channel, thus permitting the entrance of bile into the pancreatic duct, where it activated the pancreatic enzymes, and autodigestion of the organ resulted.

Clinical and experimental evidence for and against this theory forms an interesting chapter, but the results of studies concerned with the presence of bile in the pancreatic ducts as a causative factor are extremely contradictory. The opposing views are supported by apparently well established evidence and by the opinions of well qualified investigators. For a more detailed consideration of this theory the reader is referred to the reports of Flexner,[4] Archibald,[5] Mann and Giordano,[6] Nordmann,[7] Dragstedt and co-workers,[8] Wolfer[9] and Wangensteen and collaborators.[10]

Reflux of Duodenal Contents.—It has been believed by some investigators that a reflux of duodenal contents into the pancreatic ducts is the responsible factor in initiating self destruction of the pancreas. Unsuccessful attempts to produce the disease by injecting duodenal contents and extracts of sterile duodenal mucosa (Quick;[11] Pólya;[12] Starling[13]) seem to discredit this theory.

Lymphatic Theory.—For many years observers have noted the frequent association of acute pancreatitis and infection of the biliary tract. In 1897 Klippel[14] mentioned the lymphatic route as the possible connection between the inflamed gallbladder and the pancreas. Later Maugeret,[15] Arnsperger,[16] Deaver and Sweet[17] and Sweet[18] accepted this explanation. As late as 1921, Judd[19] believed in this route of the spread of infection from the region of the biliary tract. However, Kaufmann[20] discussed the lymphatic theory in an excellent manner and showed by experiments on rabbits that a lymphatic route of infection to the pancreas is unlikely. The facts that hemorrhagic pancreatitis has not been produced experimentally by this method, that no proof has been offered to permit the acceptance

3. Opie, E. L.: Bull. Johns Hopkins Hosp. **12**:182, 1901.
4. Flexner, S.: J. Exper. Med. **8**:167, 1906.
5. Archibald, E.: Surg., Gynec. & Obst. **28**:529, 1919.
6. Mann, F. C., and Giordano, A. S.: Arch. Surg. **6**:1, 1923.
7. Nordmann, O.: Chirurg **1**:721, 1929.
8. Dragstedt, L. R.; Haymond, H. E., and Ellis, J. C.: Arch. Surg. **28**:232, 1934.
9. Wolfer, J. A.: Internat. S. Digest **7**:211, 1929.
10. Wangensteen, O. H.; Leven, N. L., and Manson, M. H.: Arch. Surg. **23**: 47, 1931.
11. Quick, B.: Australian & New Zealand J. Surg. **2**:115, 1932.
12. Pólya, E. A.: Berl. klin. Wchnschr. **43**:1562, 1906.
13. Starling, H. J.: Guy's Hosp. Rep. **82**:269, 1932.
14. Klippel, M.: Arch. gén. de méd. **180**:536, 1897.
15. Maugeret, R.: Cholécystopancréatite; essai de pathogénie, Thesis, Paris, no. 300, 1908; cited by Kaufmann.[20]
16. Arnsperger, L.: München. med. Wchnschr. **58**:729, 1911.
17. Deaver, J., and Sweet, J. E.: J. A. M. A. **77**:194, 1921.
18. Sweet, J. E.: Internat. Clin. **4**:293, 1915.
19. Judd, E. S.: J. A. M. A. **77**:197, 1921.
20. Kaufmann, M.: Surg., Gynec. & Obst. **44**:15, 1927.

of this explanation on anatomic grounds and that attempts to culture organisms have failed in most cases of pancreatic necrosis speak against this theory.

Vascular Theory.—The opinion that hemorrhagic pancreatitis may result from some circulatory disturbance is not new. Because of our special interest in this theory, the significant contributions in the literature relative to vascular lesions in the pancreas are reviewed in this report.

Panum,[21] as early as 1862, injected small particles of wax into the pancreatic arteries, thus producing hemorrhage in the pancreas. Later Lépine[22] injected lycopodium powder into the chief arteries of the pancreas and after a few hours observed hemorrhagic infarcts, with death occurring subsequently. Bunge[23] produced hemorrhagic infarcts regularly by first ligating the artery and then injecting air, petroleum or some other oil. With simple ligation of the arteries the animals did not die, but after ligation and intra-arterial injection (especially of oil) the animals died in a few days and the pancreas showed blue-black infarcts.

One of the earliest experimental methods for the production of acute pancreatic necrosis was the mass ligation of the vessels to and from the pancreas. This was frequently done simultaneously with transecting the gland at one or more levels. Langerhans,[24] in 1890, was the first to produce the disease by this method. Later Hildebrand,[25] by crushing and by double ligation of the gland, was able to produce pancreatic necrosis. He demonstrated for the first time the relationship between pancreatic disease and fat necrosis. Katz and Winkler,[26] Doberauer[27] and Levin[28] all found that severe mechanical damage, i. e., crushing, with ligation of most of the vessels, resulted in pancreatic and fat necrosis. Levin concluded that the greatest effect occurred in those animals in which there was the most interference with the blood supply to the organ.

Blume,[29] in 1897, was the first to cause this disease by using digital compression to alter the blood supply. He held the pancreatic vessels in cats for ten minutes and thus produced acute ischemia. Small foci of necrosis resulted. His explanation for this necrosis was that the returning circulation was not sufficient to save the gland cells which were altered by the short interruption of oxygen, and that the ferments, becoming activated, caused local softenings. Beneke,[30] referred to these observations and remarked that the necrosis resulting from the vascular damage underlay the specific autodigestion seen in acute pancreatitis. Milisch,[31] Lewit[32] and Wulff[33] also reported acute pancreatitic necrosis resulting

21. Panum, P. L.: Virchows Arch. f. path. Anat. **25**:308, 1862.
22. Lépine, R.: Rev. de méd. **12**:402 and 481, 1892.
23. Bunge: Arch. f. klin. Chir. **71**:725, 1903.
24. Langerhans, R.: Virchows Arch. f. path. Anat. **122**:252, 1890.
25. Hildebrand: Zentralbl. f. Chir. **22**:297, 1895.
26. Katz, A., and Winkler, F.: Arch. f. Verdauungskr. **4**:289, 1898.
27. Doberauer, G.: Beitr. z. klin. Chir. **48**:456, 1906.
28. Levin, I.: J. M. Research **16**:419, 1907.
29. Blume, F.: Zur Frage der intravitalen Selbstverdauung des Pankreas, in Beiträge zur wissenschaftlichen Medicin. Festschrift dargeboten den medicinischen Theilnehmern an der LXIX. Versammlung deutscher Naturforscher und Aerzte vom herzoglich braunschweigischen Staatsministerium, Brunswick, 1897, p. 132.
30. Beneke, R.: München. med. Wchnschr. **78**:1773, 1931.
31. Milisch, O.: Experimenteller Beitrag zur Lehre von dem Zusammenhang entzündlicher Pankreaserkrankungen mit Nekrosen des Fettgewebes, Berlin, E. Ebering, 1897.
32. Lewit, cited by Kummer, R. H.: Schweiz. med. Wchnschr. **57**:513, 1927.
33. Wulff, P.: Berl. klin. Wchnschr. **39**:734, 1902.

from local ischemia. Zenker,[34] in 1874, reported a clinical picture that he had observed three times and designated it "pancreatic apoplexy." He established the theory that the extravasation of blood about the pancreas or the swelling of the gland due to edema or hemorrhage produced a nervous reflex on the solar plexus and thereby a condition of lethal shock. Numerous investigators have attempted to explain the development of the disease on the basis of a similar nervous reflex, but acting on the pancreatic arteries rather than on the solar plexus. Knape[35] injected various substances containing proteolytic ferments into the pancreas and found that stasis and hemorrhagic infarction occurred. He believed that trypsin acts as a powerful stimulus to the nerves of the blood vessel walls.

Beneke[30] and Brütt[36] adhered to the theory of a nervous reflex mechanism. Marcus,[37] a strong advocate for vascular disturbances in the establishment of the disease, thought that the nervous reflexes acting on the vessels account for the condition when it occurs immediately after a big meal, an operation, trauma or poisoning and when there is no lesion in the bile passage. He wrote, "We will arrive at a satisfactory explanation of all cases of pancreatitis if we consider investigations which regard the breakdown of pancreatic tissue as the primary cause of pancreatic necrosis and as secondary to this, the activation of the ferment."

The first account of pancreatic infarcts was by Brentano[38] in 1900. Körte[39] reported the case of an obese woman, 55 years of age, who experienced symptoms of acute pancreatitis following a fatty meal and died twenty hours later. At autopsy there was a half-liter of hemorrhagic fluid in the abdominal cavity, and the pancreas showed marked swelling, with two infarcts, which occupied fully one third of the entire organ. Rössle[40] observed occasional anemic pancreatic infarcts, and Gerlei,[41] studying the pancreas in 30 cases of heart disease, found thrombi in the vessels in 4 cases; in 1 instance he found severe arteriosclerosis, thrombosis of the splenic artery and an infarct of the pancreas.

Löwenthal[42] reported changes in the arteries which favored a vascular factor in the causation of acute pancreatic necrosis. In 15 cases he found, at autopsy, necrosis of the media in the smaller arteries, either involving the entire circumference or limited to only a small portion. The significance of this lesion, he pointed out, is that blood stagnates in the necrotic dilated regions and therefore the blood supply to the areas supplied by these vessels is reduced or even interrupted. Thus death may occur even without arterial occlusion.

Rich and Duff,[43] in an excellent contribution on the problem of acute pancreatitis, indicated the importance of the alterations of circulation in the destruction of the parenchyma in this disease. In a convincing manner, they presented experimental evidence to prove that trypsin is the substance responsible for pancreatic necrosis. This enzyme is liberated from the pancreatic duct system

34. Zenker, F. A.: Deutsche Ztschr. f. prakt. Med. **1**:351, 1874.

35. Knape, W.: Virchows Arch. f. path. Anat. **207**:277, 1912.

36. Brütt, H.: Virchows Arch. f. path. Anat. **246**:33, 1923.

37. Marcus, M.: Beitr. z. klin. Chir. **149**:129, 1930.

38. Brentano, A.: Arch. f. klin. Chir. **61**:789, 1900.

39. Körte, W.: Die chirurgischen Krankheiten und die Verletzungen des Pankreas, in Billroth, T., and Luecke, A.: Deutsche Chirurgie, Stuttgart, Ferdinand Enke, 1898, pt. 45 d; cited by Bunge.[23]

40. Rössle, R.: Beitr. z. path. Anat. u. z. allg. Path. **69**:163, 1921.

41. Gerlei, F.: Virchows Arch. f. path. Anat. **276**:148, 1930.

42. Löwenthal, K.: Deutsche med. Wchnschr. **58**:1209, 1932.

43. Rich, A. R., and Duff, G. L.: Bull. Johns Hopkins Hosp. **58**:212, 1936.

(e. g., in obstruction by epithelial metaplasia or by calculous material) and acts on the arterial wall causing necrosis. This necrosis is followed by either thrombosis or hemorrhage. The mechanical tearing of the gland by hemorrhage tends to open up still further the excretory pancreatic duct system, permitting the escape of more pancreatic juice and thus favoring the development of further thrombosis. They contended that this spreads progressively throughout a large area of the gland.

In their report a vascular lesion which they found in all of their cases of acute hemorrhagic pancreatitis is described as follows: "The adventitia of the affected artery may appear condensed and pink-stained and may contain leukocytes or necrotic cells; but the striking changes are in the media. The muscular fibers of the media swell, and are sometimes separated by fluid or by spaces in which nothing stains; their nuclei become shrunken, pyknotic and often karyorrhectic and polymorphonuclear cells may be found in the lesion in the early stages. . . . Individual fibrils split off so that the elastic membrane appears frayed. . . . Finally all nuclear staining is lost and the necrotic tissue of the vessel walls stains homogeneously pink with eosin. The first alterations in the media are always found in the outer layer, the muscle fibers of which may be necrotic, while those near the intima remain intact, but the damage in most cases proceeds rapidly to involve the entire thickness of the vessel wall, or may involve the entire circumference. Complete necrosis and rupture of the wall of an artery may occur in a small segment of its circumference, while the remainder maintains a perfectly normal appearance in structure. Destruction of a segment of the vessel wall was most frequently found in the larger vessels, while destruction of the whole circumference was the rule in the smaller vessels. When the smaller arteries and arterioles have become necrotic or hyalinized their appearance is often quite indistinguishable from that characteristic of the familiar arteriosclerosis occurring in man in association with hypertension and arteriosclerotic nephritis." These authors regard these changes as the "specific vascular lesion" of acute hemorrhagic pancreatitis.

In spite of the evidence which implicates circulatory abnormalities, many recent writers (Baló;[44] Bernhard;[45] Popper;[46] Dragstedt[8]) have felt that the vascular processes play only a minor part in the etiology of acute hemorrhagic pancreatitis, ranking this group among such rare causes as ascarides invading the pancreatic duct, trauma, duodenal diverticula or acute infectious diseases.

Thus it seems evident from a study of the various theories which have been presented to explain the mechanism of the production of this disease that the lymphatic theory and the theory of the reflux of duodenal contents are without sufficient clinical or experimental support. The "common channel" theory cannot satisfactorily explain any large group of cases. Our review indicates that the vascular theory is upheld by many reports, both of experimental work and of the postmortem observations in patients who have died of the disease. Therefore, this hypothesis as to the cause of acute hemorrhagic necrosis is worthy of further consideration.

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44. Baló, J.: Beitr. z. path. Anat. u. z. allg. Path. **92**:14, 1933.
45. Bernhard, F.: Deutsche med. Wchnschr. **61**:667, 1935.
46. Popper, H. L.: Wien. klin. Wchnschr. **47**:295, 1934.

SUMMARY OF POSTMORTEM OBSERVATIONS IN FORTY CASES

Forty cases of acute hemorrhagic pancreatitis that were studied in the department of pathology of the University of Michigan between the years 1897 and 1937 were accepted for inclusion in this report. All of them afforded microscopic evidence of pancreatic necrosis and hemorrhage. Purulent infiltrations were not considered essential but were present in some instances. The number of slides available for study in each case varied from one to eight, with an average of five sections per case. In many of the older records clinical data were lacking, and in some instances only a limited autopsy had been permitted.

The age was recorded in 32 of the 40 cases. The youngest patient was a premature infant, and the oldest, a man of 78 years. The average age was 46 years. Of the 36 patients whose sex was recorded, 22 were female.

Our attention was directed not only to the vascular changes in and about the pancreas but also to the alterations in the blood vessels in the more distant organs. Thrombosis was the most frequently observed vascular lesion; it was present in 26 of the 40 cases. The veins were more frequently occluded than the arteries (fig. 1). Thrombi in stages of organization, as well as recent thrombi, were found. In 8 instances the lumens were obstructed by mature connective tissue. In one medium-sized artery there was canalization. In those cases with well organized thrombi the vascular changes were obviously older than the acute disease, and although they may have been contributing factors in the spread of the disease, they could not have been considered as precipitating factors. Their presence is significant as indicating a liability to vascular occlusion, which may have been a continuous or an intermittent feature in any particular case.

In some sections small arteries containing bland thrombi were observed with no necrosis of the pancreas about these arteries, while in other sections the occluded arteries lay immediately adjacent to a small area of necrosis and hemorrhage. If serial sections had been available, it might have been shown that such thrombi were in continuity with the area of necrosis. From the material available it could not be determined whether the fresh thromboses occurred before or after the establishment of necrosis.

There were only 2 cases in which emboli were found in pancreatic arteries, and in 1 of these the autopsy was limited to the abdomen. In 1 additional case (no. 31) there were mural thrombi in the left and right ventricles of the heart, with multiple infarcts in the lungs, myocardium and kidneys, but no pancreatic embolus was found.

Hemorrhage into the pancreatic substance was so prominent in 3 cases that the process was considered to be essentially one of pancreatic

infarction and could be termed "pancreatic apoplexy." In other cases, in which hemorrhage was less extensive, it was found along the interlobular septums, separating the lobules and surrounding the acini. The lobules adjacent to the extravasated blood were generally normally preserved. In many places, however, there was necrosis of the periph-

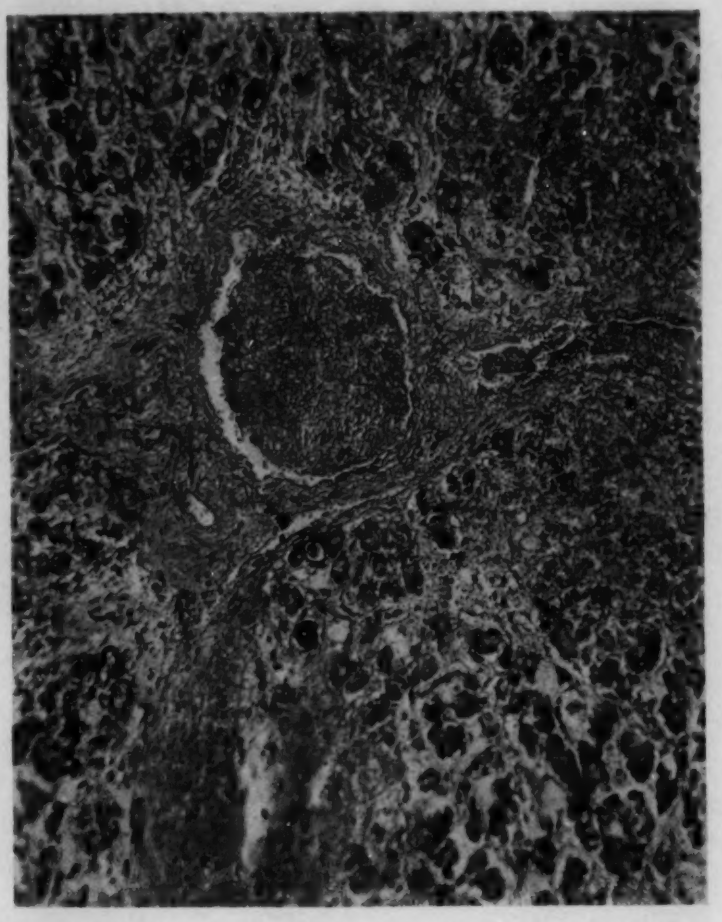

Fig. 1 (case 9).—A small artery and vein, the vein almost completely occluded by an organized thrombus. In the adjacent pancreatic lobules are necrosis, hemorrhage and edema. Hemalum and eosin; × 160.

eral portion of the lobule while the central area remained normal. The location of this necrotic zone indicated that the necrosis might have resulted either from an impairment of circulation or from the spread of a toxic agent with the hemorrhage.

Necrosis of pancreatic parenchyma was not limited by, or confined to, any anatomic boundaries. Necrotic areas varied in size from a few acini to almost the entire organ. Along the border of regions of extensive necrosis were found lobules which were in part necrotic and in part normal. Extensive areas of necrosis without hemorrhage were common,

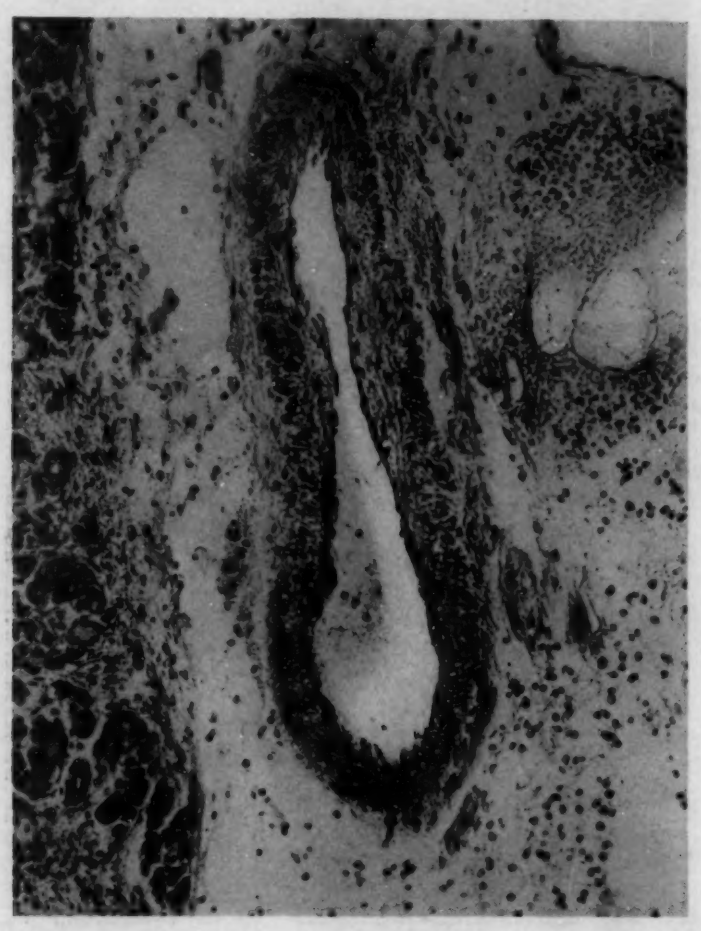

Fig. 2 (case 20).—An interlobular artery with necrosis through the entire thickness of the wall along one fourth of its circumference. Hemalum and eosin; × 180.

indicating that the escape of blood was prevented by thrombosis antedating necrosis.

In each case the sections were examined for the presence of necrosis, edema, inflammatory infiltrations and hemorrhage in the walls of arteries and veins. In 11 of the 40 cases small lesions were found which cor-

responded to the vascular lesions described by Rich and Duff [48] and claimed by them to be specific for this disease. Figures 2, 3 and 4 illustrate these vascular changes.

Because of the frequently reiterated theory that obstruction of the pancreatic duct with subsequent rupture of the duct is the factor responsible for the disease, it is of interest that in this series a stone was found

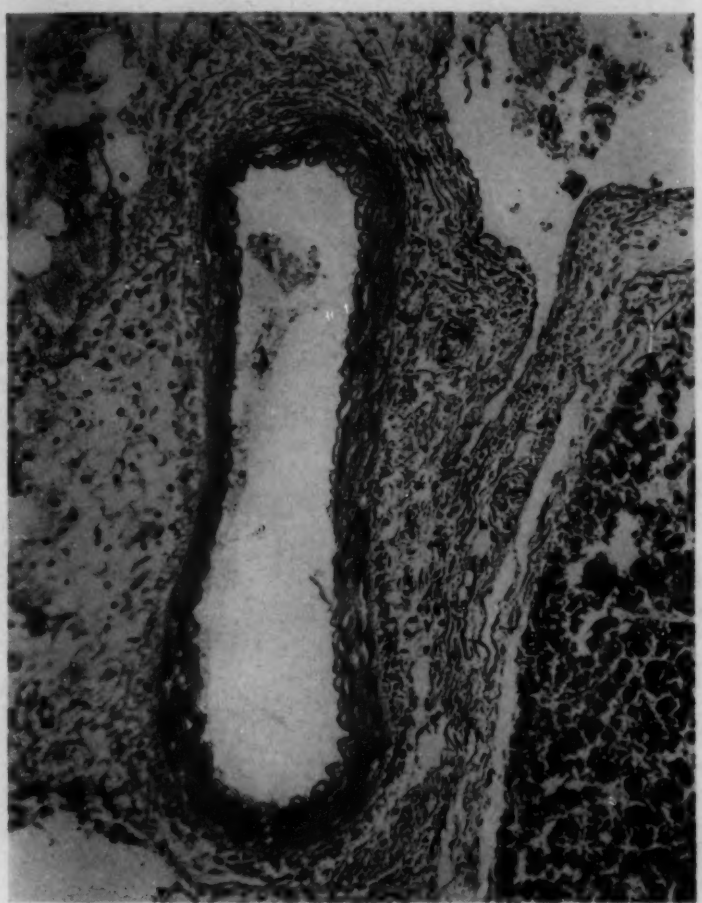

Fig. 3 (case 32).—Necrosis of an artery involving one half of the circumference and extending through all coats. Hemalum and eosin; × 180.

occluding the ampulla of Vater in only 1 case. Obstruction of the pancreatic duct due to metaplasia of ductal epithelium was also looked for, because it likewise has been postulated to account for acinar rupture and escape of pancreatic juice. In 13 of the 40 cases of this series, or 33 per cent, epithelial metaplasia was found.

It seems evident from a review of these 40 cases that any conclusions regarding the genesis of pancreatic necrosis based on a study of autopsy material must at best be speculative, for one is dealing with end stages. Evidence of the responsible factors may well have been destroyed early in the course of the disease, without leaving a trace. Various circulatory

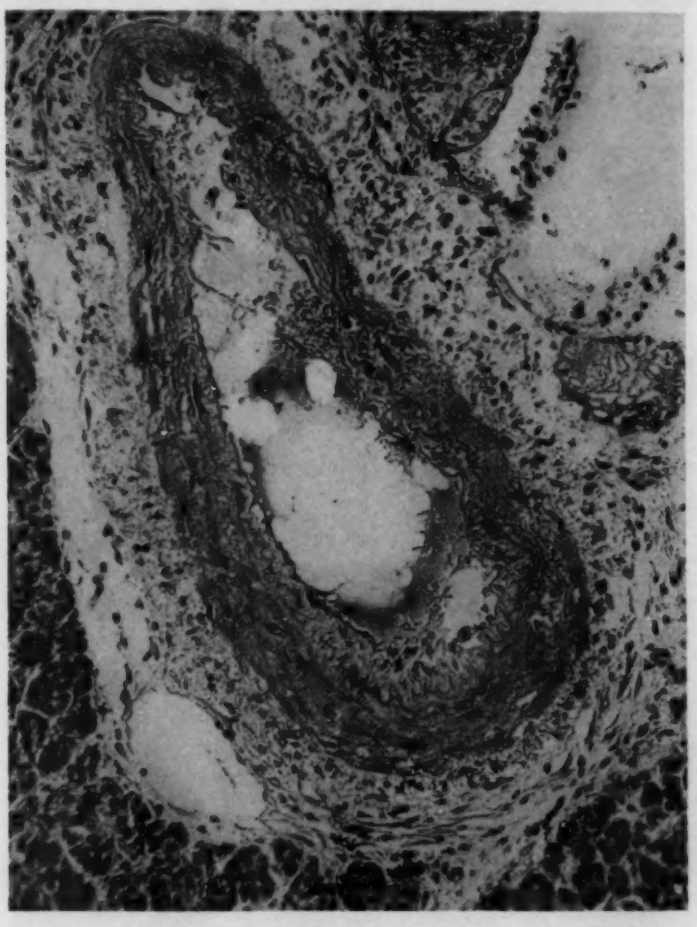

Fig. 4 (case 22).—An artery with widespread necrosis, edema and hemorrhage. The clearly defined internal elastic lamina of the normal segment is no longer visible in the necrotic segment. Hemalum and eosin; × 180.

changes in the pancreas have been seen, but there is no constant relationship between the type of vascular change found in distant organs and those found in and about the pancreas. In 3 instances the process in the pancreas appeared to be essentially one of infarction, but after a careful review of these cases I am not able to demonstrate that the necrosis

followed a vascular occlusion. Even though in many instances there were extensive circulatory changes there is no conclusive proof that the pancreatic necrosis is secondary to these vascular changes.

The mechanical factors which have been postulated, such as calculus, were seldom present in my cases. The finding of a stone in the ampulla of Vater in only 1 case in 40 corresponds favorably to the reports from other clinics. For example, Guleke [47] with a series of 437 cases of acute pancreatic necrosis found a stone blocking the common outlet in only 1.4 per cent. Schmieden and Sebering [48] with a series of 1,278 cases collected from the various German clinics noted the presence of a stone in 4.4 per cent. From the English and American literature I have collected 245 cases of this disease in which mention has been made of the presence of a calculus and among these have found 32 cases, or 13 per cent, in which the stone blocked the common duct.

A complete solution for the problem of the genesis of this malady will probably not be found in a study of material obtained at autopsy, although considerable evidence of antecedent vascular lesions was found in the present series. I therefore undertook the experimental production of acute pancreatic necrosis in order to study the vascular changes in their earliest stages and to obtain additional evidence for, or against, the vascular theory.

EXPERIMENTAL PRODUCTION OF ACUTE PANCREATIC NECROSIS

In a series of 21 dogs an attempt was made to produce the disease by injecting small droplets of metallic mercury into one of the pancreatic arteries. By thus inducing embolism, infarcts were produced when the pancreas was in various stages of secretory activity.

Method.—Healthy dogs, weighing between 15 and 18 Kg., were kept in metabolism cages. Operative anesthesia was obtained by injecting intravenously sodium pentobarbital in doses of 1 grain (0.065 Gm.) per kilogram of body weight. The dogs were divided into three groups. Five dogs (group 1) received injections of mercury after a fast of eighteen hours. Twelve dogs (group 2) were fed a prepared protein and a cereal dog food [48a] two hours before the operation. Six dogs (group 3) were given subcutaneous injections of acetylbetamethylcholine chloride in doses of 0.5 mg. per kilogram of body weight, together with 1 mg. of physostigmine salicylate, one hour before the operation.

Using sterile surgical technic, an incision 16 cm. long was made in the right upper quadrant of the abdomen. By displacing the omentum, the pyloric end of the stomach and the duodenum were easily identified and the duodenum and the anterior portion of the pancreas were brought through the incision. In the dog the pancreas is a V-shaped organ with its apex at the pylorus of the stomach and with

47. Guleke, N.: Berl. klin. Wchnschr. **41**:682, 1904.

48. Schmieden, V., and Sebering, W.: Surg., Gynec. & Obst. **46**:735, 1928.

48a. This food is marketed as the Red Heart brand. The formula is: protein, 10.5 per cent; fat, 2.5 per cent; fiber, 1 per cent.

an anterior ramus lying parallel to, and in the mesentery of, the first portion of the duodenum. A small artery was found to be of constant occurrence, supplying the tip of this portion of the pancreas and sending a few branches to the duodenum. Using a curved no. 26 hypodermic needle on a tuberculin syringe, droplets of sterile metallic mercury were forced into the lumen of this small artery. Digital compression of the branches to the duodenum was found necessary to prevent emboli from lodging there and producing infarcts of the intestinal wall. In a few instances attempts to make injections into the artery supplying the tip were unsuccessful. In such instances a larger artery near the apex was chosen. Particles of mercury could be seen going into the branches supplying the pancreas. The amount injected varied from 2 to 10 cu. mm. No significant hemorrhage resulted after the needle was withdrawn from the artery. Following the operation, the laparotomy incision was closed without a drain, and the dogs were placed in metabolism cages. After recovery from the anesthesia, the consumption of food and water was not restricted, and the dogs were killed between the third and twenty-ninth postoperative days.

Fig. 5 (dog 2).—Roentgenogram of the extirpated pancreas, with the attached duodenum held by hemostats. Mercury has been injected into many of the arteries of the duodenal ramus of the pancreas. A few particles of mercury have entered arteries in the wall of the duodenum.

The pancreas was removed as soon after death as possible and placed in a 10 per cent dilution of solution of formaldehyde U. S. P. A roentgenogram was made of each extirpated pancreas to localize the droplets of mercury (fig. 5). Serial blocks were selected from the infarcted portions and additional blocks from the more normal areas of the pancreas. The sections were stained with hematoxylin and eosin.

Results.—A summary of the results is given in the table. Mercury was successfully injected into the arteries supplying the pancreas in 19 of the 21 dogs. In 4 of the 19, however, embolism resulted also in branches supplying the duodenum, and these dogs (3, 8, 10 and 18) died from generalized peritonitis following perforation of infarcted areas

of the intestinal wall. Two dogs (9 and 11) failed to recover from the anesthesia and died within two hours after the injection. One died of lobular pneumonia on the fifth day after the operation.

Emboli of Metallic Mercury in Pancreatic Arteries of Dogs

Dog	Injection Satisfactory	Death, Days or Hours After Operation	Mode of Death	Pancreatic Fat Necrosis	Fluid in Abdomen	Perforation of Duodenum	Comment
Group 1.	No Stimulus						
1	Yes	3 days	Killed	Yes	No	No	Acute hemorrhagic pancreatitis
2	No	3 days	Killed	Yes	No	No	Acute interstitial pancreatitis
3	Yes	5 days	Killed	Yes	Yes	Yes	Acute hemorrhagic pancreatitis; generalized peritonitis
4	No	29 days	Killed	No	No	No	Small area of interstitial pancreatitis
5	Yes	5 days	Found dead	No	No	No	Bronchopneumonia
Group 2.	Food as Stimulus						
6	Yes	11 days	Killed	Yes	No	No	Chronic interstitial pancreatitis
7	Yes	15 days	Killed	Yes	No	No	Chronic abscess of pancreas
8	Yes	22 hours	Found dead	Yes	Yes	Yes	Acute hemorrhagic pancreatitis; generalized peritonitis
9	Yes	2 hours	Found dead	No	No	No	Death from anesthesia
10	Yes	20 hours	Found dead	Yes	Yes	Yes	Acute hemorrhagic pancreatitis; generalized peritonitis
11	Yes	2 hours	Found dead	No	No	No	Death from anesthesia
12	Yes	12 days	Killed	Yes	No	No	Abscess of pancreas
13	Yes	7 days	Killed	Yes	No	Yes	Acute hemorrhagic pancreatitis; localized peritonitis
14	Yes	11 days	Killed	Yes	No	No	Chronic abscess of pancreas
15	Yes	14 days	Killed	Yes	No	No	Small chronic abscess of pancreas
Group 3.	Acetylbetamethylcholine Chloride						
16	Yes	9 days	Killed	No	No	No	Abscess of pancreas
17	Yes	8 days	Killed	Yes	No	No	Abscess of pancreas
18	Yes	26 hours	Found dead	No	Yes	Yes	Acute hemorrhagic pancreatitis; generalized peritonitis
19	Yes	5 days	Killed	No	No	No	Acute hemorrhagic pancreatitis; small abscess of pancreas
20	Yes	9 days	Killed	No	No	No	Chronic interstitial pancreatitis
21	Yes	9 days	Killed	Yes	No	No	Subacute hemorrhagic pancreatitis

In every case in which the intra-arterial injection was limited to the pancreas, the animal recovered from the operation completely and within forty-eight hours was eating and drinking normally. In no instance

could death be attributed to acute hemorrhagic pancreatitis. Daily preoperative and frequent postoperative determinations of urinary diastase were made in groups 2 and 3, and the results have been reported elsewhere.[49]

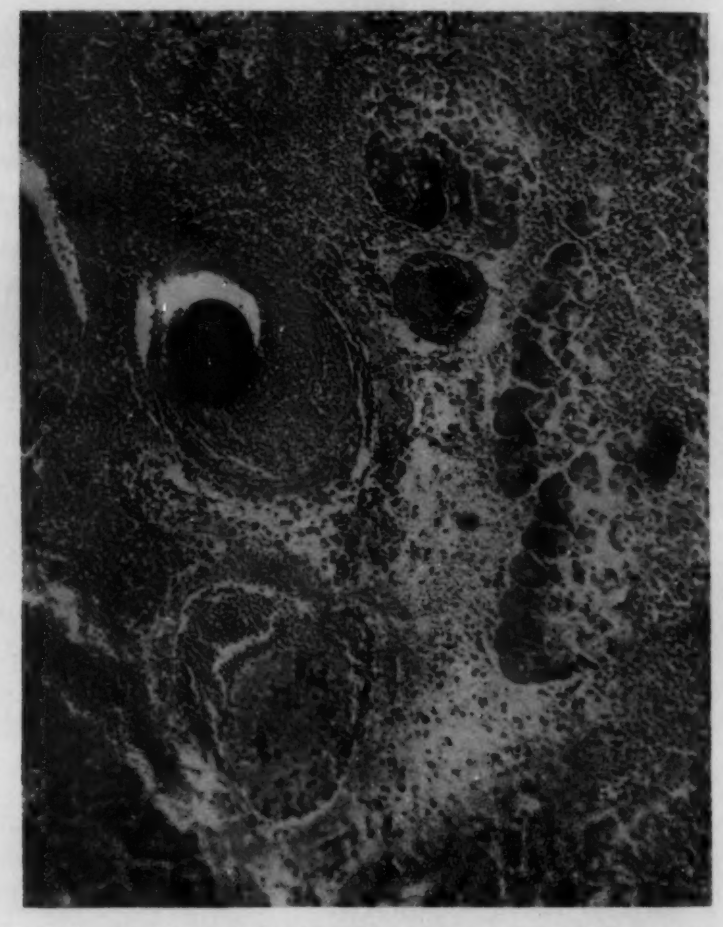

Fig. 6 (dog 10).—An embolus of metallic mercury partly occludes a small artery. The remainder of the lumen of the artery and the adjacent vein are thrombosed. In the surrounding tissue are leukocytic infiltration, edema, hemorrhage and necrosis of pancreatic acini. A few normal acini are still present. Hemalum and eosin; × 160.

Gross Observations.—When the abdomens of the 5 dogs with duodenal perforation were opened, all but 1 were found to have free

49. Smyth, C. J.: Ann. Int. Med. **12**:932, 1939.

fluid in the peritoneal cavity, with generalized acute fibrinopurulent peritonitis and marked hemorrhage, edema and pancreatic fat necrosis of the pancreas and of other structures immediately adjacent to it. In all cases in which the emboli occluded only pancreatic vessels the resulting parenchymal lesion remained localized to the area deprived of its blood supply, and the necrosis did not spread to adjacent noninfarcted pancreatic tissue. In those animals killed between the third and fifth days the omentum, mesentery and duodenum were found to be drawn up over and about the infarcted area by fibrinous exudate, thus walling it off from the more normal structures. After the ninth to the eleventh day firm fibrous bands were found about the tip of the pancreas. Fat necrosis was seen in 13 of the 21 dogs. In 7 of the 14 animals examined between the fifth and fifteenth days small pancreatic abscesses, varying from 2 to 4 cm. in diameter, had developed. The walls of these abscesses were formed by dense fibrous tissue. In dogs examined between the ninth and twenty-ninth days the tip of the pancreas was very firm and bound to the neighboring structures by fibrous adhesions. When cut across the tip, it appeared to be replaced by scar tissue.

Microscopic Observations.—In all of the 19 cases in which intra-arterial injection was successful there were hemorrhage and pancreatic necrosis. In specimens obtained between twenty and seventy-two hours after the production of infarction there were the typical changes of acute hemorrhagic pancreatitis, namely, edema, hemorrhage, necrosis of pancreatic parenchyma and of fat, and polymorphonuclear infiltrations (fig. 6). In many of the small arteries the lumens were partially or completely occluded by rounded particles of mercury (fig. 7). Frequently there was no inflammatory reaction in arterial walls immediately in contact with the metal, thus excluding chemical irritation as a causal agent. Arteries occluded by mercury were found in sections without evidence of pancreatitis and also in areas where there was necrosis of entire lobules. In many instances only a residue of the necrotic arterial wall remained about the rounded embolus of mercury.

Another change seen frequently in the early cases (up to seventy-two hours) was necrosis of the central portion of an entire lobule with a marginal strip entirely normal (fig. 8). In the area between this necrotic center and the normal peripheral portion the cells of the acini had lost their normal acidophilic granules and the nuclei were closely packed together.

By the fifth day there was well marked fibroblastic and angioblastic proliferation at the border of each area of infarction. In the walls of larger areas of necrosis, in which suppuration and abscesses developed, there was replacement of acinar tissue by fibrous connective tissue. In the few animals that were permitted to live after the fourteenth day the

areas of ischemic necrosis were replaced by scar tissue. In about 50 per cent of our dogs changes were found in the vessel walls which could be considered similar to the specific vascular lesion of Rich and Duff,[43] but the vessels thus involved were always in or near areas of infarction.

Other investigators have used various embolic agents for the production of experimental pancreatitis, but they have given no attention to the

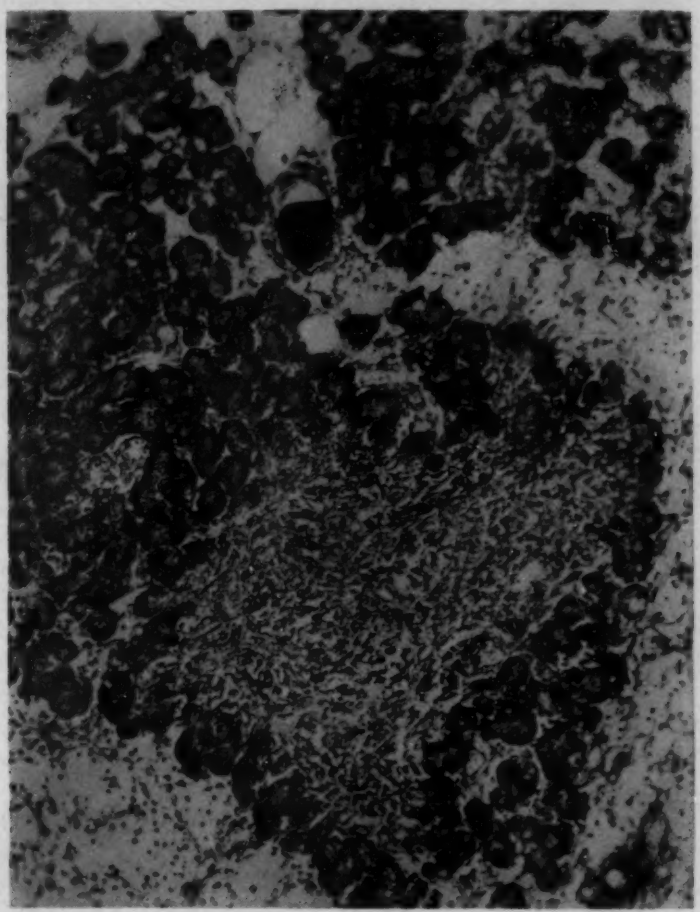

Fig. 7 (dog 11).—A small arteriole is practically filled with mercury. In an adjacent pancreatic lobule the central acini are replaced by fibrous connective tissue, a healing infarct. The peripheral acini of this lobule survived. Hemalum and eosin; × 160.

stage of the glandular activity at the time infarction was produced. Clinically, it has been observed frequently that the onset of the disease is at the acme of pancreatic secretion. Therefore, it was thought that if

focal areas of pancreatic necrosis could be produced at a time best suited for the liberation of concentrated digestive enzymes, optimal conditions would exist for inducing a spreading self destruction of the gland. The

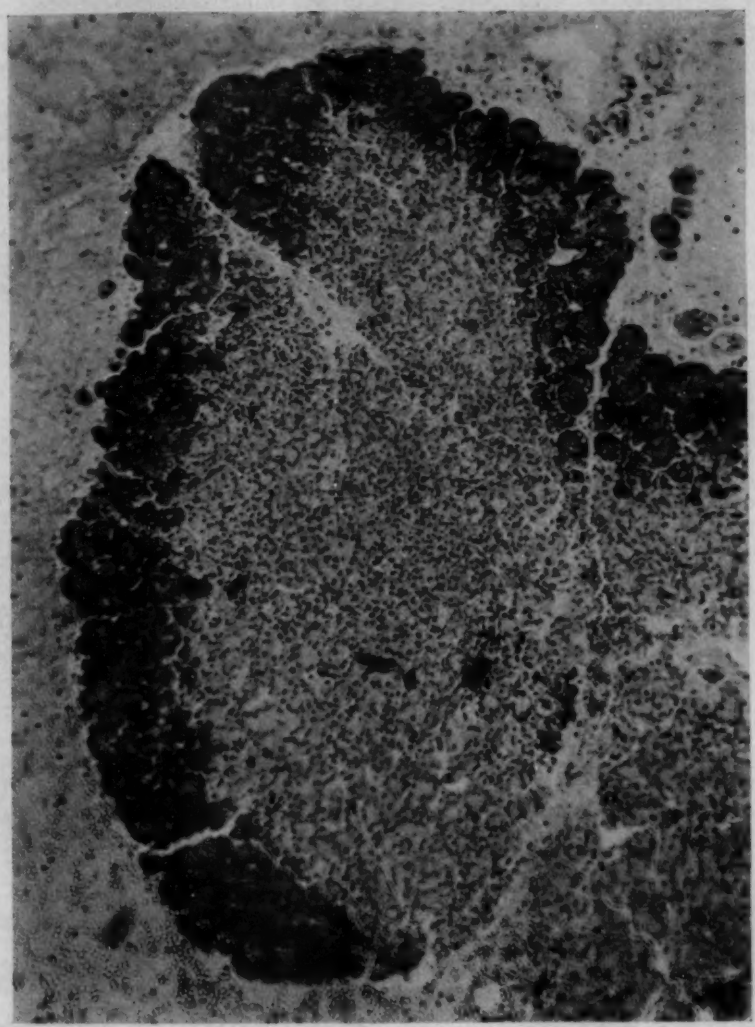

Fig. 8 (dog 10).—A pancreatic lobule showing central acinar necrosis, with the acini at the periphery living. There are interlobular edema and hemorrhage. Hemalum and eosin; × 160.

use of acetylbetamethylcholine chloride and physostigmine to augment secretory activity was decided on because Friedman and Thompson [50]

50. Friedman, I., and Thompson, W. R.: Ann. Surg. **104**:388, 1936.

had shown that these drugs when given together are powerful stimulants to pancreatic excretion. As shown by group 3 in the table, no significant modification of the results followed this procedure.

In the experiments with dogs it was impossible to produce fatal pancreatic necrosis, regardless of the state of functional activity. In each instance it was observed that the resulting pancreatitis was sharply limited to the area deprived of its blood supply. In these animals, with the capsule of the organ intact, the pancreas showed marked ability to repair local damage. This demonstration of the ability of the dog's pancreas to undergo spontaneous healing is compatible with the clinical observations of Elman,[51] who described acute nonhemorrhagic pancreatitis. It is therefore not improbable that in many cases unexplained pain in the upper regions of the abdomen may be due to acute pancreatic disease.

A study of the urinary diastase values was made in 16 dogs of this series, as an index to the presence of acute pancreatic necrosis. Various observers (Nordmann;[7] Rost;[52] Foged;[53] McCaughan[54]) have indicated that with the aid of laboratory tests for diastase it is possible to establish a preoperative diagnosis of acute hemorrhagic pancreatitis in 85 per cent of the cases. I have used a modification of the Wohlgemuth test.

Opportunity for a satisfactory series of liastase studies was present in 11 of the 16 dogs. In 8 of the 11 animals for which an adequate number of specimens was examined, urinary diastase was significantly elevated. This series was small, but it was controlled by autopsy studies, and in all cases there was evidence of pancreatic necrosis.

SUMMARY

No one of the current theories of the genesis of acute hemorrhagic pancreatitis adequately explains the mechanism of the production of the disease.

There is abundant evidence in the literature to show that vascular disturbances play an important etiologic role in many instances.

It seems evident that the solution to the problem of the genesis of this disease will be found in experimental studies in which the earliest stages of the disease can be investigated, rather than in review of material obtained at autopsy.

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51. Elman, R.: Am. J. Digest. Dis. & Nutrition **4**:732, 1938.
52. Rost, F.: München. med. Wchnschr. **80**:1971, 1933.
53. Foged, J.: Am. J. Surg. **27**:439, 1935.
54. McCaughan, J. M.: Surg., Gynec. & Obst. **59**:598, 1934.

Localized areas of acute pancreatic necrosis were uniformly produced in 21 dogs by injecting mercury into the pancreatic artery, but none of these animals died of a spreading type of acute pancreatic necrosis. The frequent production of the "specific" vascular lesion described by Rich and Duff [43] indicates that this lesion is a result of, or concomitant with, the disease; it is not of etiologic significance.

In these dogs small areas of pancreatic necrosis remained localized even though the adjacent normal acini were stimulated to maximum secretory function by food or by acetylbetamethylcholine chloride and physostigmine.

A COMPONENT OF GALLSTONES INSOLUBLE IN ORDINARY SOLVENTS AND ACCOUNTING IN PART FOR THEIR DARK COLORATION

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Owing to lack of information on the chemical composition of gallstones, their classification in the literature is frequently based mainly on gross observation. Chemical analysis beyond that for cholesterol and calcium, because of the limitations of available methods, has contributed little to this classification.

There is still great confusion in the terminology of certain important chemical aspects. Stones in which the presence of bilirubin and calcium has been established are supposed to be composed of "bilirubin-calcium" (Romang;[1] Rous and co-workers[2]). Chemically, no proof is given that such a compound exists. Similarly, stones containing much cholesterol are described as "pure cholesterol stones," though it has not been proved that other constituents are absent. "Pure pigment stone" is one of the worst misnomers, as quantitative estimations of bile pigments are especially unreliable, and such a designation is often made because of the high color of the stone. Authors usually annotate that "pure" stones contain other substances in small amounts and that they are only relatively pure stones.

We[3] have been able to furnish some semiquantitative figures on the best known and most frequently encountered constituents of gallstones, namely, cholesterol, calcium, phosphorus and pigment. In carrying out these analyses a black residue was obtained which was insoluble in the ordinary solvents and which amounted to 7.5 to 67.8 per cent (average of thirty-four analyses, 26.5 per cent) of the original weight of the stones. It is the purpose of the present study to provide more information on this unaccounted fraction in gallstones.

From the Department of Surgery of the University of Chicago.

This work was done under a grant from the Douglas Smith Foundation.

1. Romang, F.: Fortschr. a. d. Geb. d. Röntgenstrahlen **35**:1199, 1927.

2. Rous, P.; Drury, D. R., and McMaster, P. D.: J. Exper. Med. **39**:72, 1924.

3. Aronson, H. G., and Hudson, J. E.: Proc. Soc. Exper. Biol. & Med. **39**: 271, 1938. Phemister, D. B.; Aronson, H. G., and Pepinsky, R.: Ann. Surg. **109**: 161, 1939.

The nuclei of stones, frequently considered as "potential centers of stone formation," have been studied by many authors. Rous and co-workers described, "calcium bilirubinate" and calcium carbonate nuclei with layers of cholesterol and "organic matter" on their surface deep in stones. Naunyn [4] expressed the belief that desquamated epithelial cells, pus cells and pigment compose the centers of stones. While Rosenow [5] obtained negative cultures from the nuclei of gallstones in 33 cases, Funke [6] found growth in bouillon in 31 cases in which the entire calculi were cultured, while in 71 the bouillon remained sterile. Gas (0.5 per cent oxygen, 6 to 7 per cent carbon dioxide and the remainder an odorless noncombustible gas) has been demonstrated in the central cavity of the gallstone in some cases by Kommerell and Wolpers.[7]

Lichtwitz [8] discussed the physical and chemical principles governing the solution and precipitation of the substances which form urinary and biliary calculi. He laid special stress on the importance of the colloid substances of normal bile which are precipitated in the inflamed gallbladder. These colloids form the framework of the calculi, on which cholesterol, bilirubin and proteins are precipitated. Similarly Kuru [9] attributed to the fibrin an important function as an organic colloidal skeleton in the stone formation. Other authors have reported the absence of protein in stones.

More complete analyses of gallstones are rather scarce. Pickens and co-workers [10] gave the following average percental composition of "mixed or combination gall stones": cholesterol, 94; calcium, 1.09; magnesium, 0.13; sodium and potassium, 0.05; carbonic acid anhydride, 1.06; phosphoric acid anhydride, 0.40; pigment (by difference), 3.26; fatty acids, trace; bile acids, none. With a view to unusual constituents, mention may be made here of a concrement composed of bilirubin, cholesterol and lecithin, reported by Kaiserling,[11] as well as of a gallstone composed of calcium palmitate, described by Cameron and White.[12] Salkowski [13] examined a gallstone from a patient and found it free from fat but containing a resinous substance that resembled fat in its solubility in ether and other solvents.

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4. Naunyn, B.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **33**:2, 1921.
5. Rosenow, E. C.: J. Infect. Dis. **19**:527, 1916.
6. Funke, J.: New York M. J. **84**:1077, 1906.
7. Kommerell, B., and Wolpers, C.: Klin. Wchnschr. **17**:1124, 1938.
8. Lichtwitz, L.: Ueber die Bildung der Harn- und Gallensteine, Berlin, Julius Springer, 1914.
9. Kuru, H.: Virchows Arch. f. path. Anat. **210**:433, 1912.
10. Pickens, M.; Spanner, G. O., and Bauman, L.: J. Biol. Chem. **95**:505, 1932.
11. Kaiserling, C.: Biochem. Ztschr. **2**:312, 1907.
12. Cameron, A. T., and White, F. D.: Am. J. M. Sc. **194**:783, 1937.
13. Salkowski, E.: Ztschr. f. physiol. Chem. **100**:259, 1917.

The constituents contained in the acid fraction of stones are better known. Calcium is found in gallstones in varying amounts, as stated by Phemister, Rewbridge and Rudisill [14] and Phemister, Day and Hastings [15] in their articles, in which they reviewed the literature on this subject. It varies from traces to almost pure calcium.

In addition to calcium and phosphorus, other metals, though present in gallstones in very small quantities, have been reported frequently. Schoenheimer and Herkel [16] demonstrated copper, zinc, manganese and iron in appreciable amounts in gallstones. Ernest Mueller [17] found, in addition to the usual metals, nickel, carbon monoxide, bismuth and tin. Ruthardt and Hirschmann [18] found more zinc in pigment stones than in other types. Spectroscopic methods were applied for analysis by Gerlach,[19] who found copper, lead, iron, manganese, silver and strontium frequently in gallstones. The same method was used by Ranganathan and De,[20] who reported the presence of sodium, potassium, calcium, magnesium, lead, phosphorus, aluminum, strontium, copper and iron.

Different opinions have been expressed about the presence of bile acids and bile salts in gallstones. While some have reported negative findings (Pickens and co-workers [10]), Salkowski [21] found in his analysis a bile acid which was probably desoxycholic acid.

EXPERIMENTAL STUDIES

Extraction of Residue.—Human gallstones, obtained in postmortem examinations and at operations, were dried in the oven at 54 C. for twenty-four hours, ground in a mortar and dried further in the oven for twenty-four hours.

In one experiment 66 Gm. of a dried and powdered mixture of stones obtained from several persons and varying in color from light to dark were used. The powder was extracted continuously with ether for twenty-four hours. The residue was then extracted with hydrochloric acid (1:6) by grinding in a mortar; the insoluble material was then extracted with 95 per cent alcohol for two hours, and then with chloroform until a colorless solution was obtained. (This usually takes seventy-two hours or longer.) Finally glacial acetic acid was used for the extraction. A brownish black residue was left which corresponded to about 4.5 per cent of the total original material extracted.

In another experiment we started out with 634 Gm. of a mixture of stones from several patients. Again ether, hydrochloric acid (1:6) and chloroform were used, but alcohol and glacial acetic acid were omitted. The percental yield of the

14. Phemister, D. B.; Rewbridge, A. G., and Rudisill, H.: Ann. Surg. **94**:493, 1931.

15. Phemister, D. B.; Day, L., and Hastings, A. B.: Ann. Surg. **96**:595, 1932.

16. Schoenheimer, R., and Herkel, W.: Klin. Wchnschr. **10**:345, 1931.

17. Mueller, E.: Biochem. Ztschr. **286**:182, 1936.

18. Ruthardt, K., and Hirschmann, H.: Centralbl. f. allg. Path. u. path. Anat. **61**:275, 1934.

19. Gerlach, W.: Verhandl. d. deutsch. path. Gesellsch. **29**:277, 1934.

20. Ranganathan, S., and De, N. K.: Indian J. M. Research **23**:237, 1935.

21. Salkowski, E.: Ztschr. f. physiol. Chem. **98**:281, 1916.

black material was somewhat larger (42.5 Gm., corresponding to 6.7 per cent), and in this residue it was possible to extract bile acids with alcohol (Pettenkofer's test, Hay's test) and a small amount of pigment with glacial acetic acid.

Amino nitrogen was determined in the hydrochloric acid filtrate, and a total of about 60 mg. was found.

Experiments on Residues.—Small amounts of the residue were treated with 2 cc. each of the following: concentrated nitric acid, concentrated sulfuric acid, concentrated hydrochloric acid, glacial acetic acid, concentrated sodium hydroxide, aqueous sodium hydrosulfite. The results were as follows:

Concentrated nitric acid dissolves the solid completely. Concentrated sulfuric acid has no apparent effect. With concentrated hydrochloric acid after one-half hour there is still a solid left, but the solution is deep brown. Glacial acetic acid has a similar effect. After one-half hour on a water bath the solution is very pale green, with the solid only slightly dissolved.

Concentrated sodium hydroxide does not dissolve the solid, but the solvent turns brown.

Sodium hydrosulfite causes a change in the color of the solid, but the solution is colorless.

Oxidation of Residue.—About 2 Gm. of the residue was suspended in 15 cc. of concentrated sulfuric acid and warmed. Then a solution of potassium permanganate was added slowly until the fluid was clear and colorless. (In some cases manganese dioxide was precipitated; in others this precipitation did not occur. When manganese dioxide was precipitated it was removed by filtration.) The clear solution was extracted with ether. The ether extract contained only a trace of an oil. The aqueous solution was made alkaline with sodium hydroxide, and an inorganic precipitate was removed (probably manganous hydroxide). The alkaline solution was then extracted with ether, and again the ether extract contained only a trace of an oil. Therefore, the oxidation products are volatile or water-soluble compounds from which no important information has been gathered.

Hydrolysis of Residue.—Attempts were made to find protein in the residue. Coagulated protein if present should be in the residue, and hydrolysis should result in the liberation of amino groups. Hydrolysis with concentrated hydrochloric acid for thirty-six hours did not cause the material to dissolve. The filtrate contained 1.1 per cent amino nitrogen, calculated on the basis of the original quantity of residue used (Van Slyke method of determination).

Enzyme hydrolysis yielded even less amino nitrogen. Dog's gastric juice liberated only traces of amino nitrogen, while solutions of either pepsin or trypsin had no apparent effect. This difference might be explained by the presence of hydrochloric acid in the gastric juice.

Chemical Analysis of Residue.—An analysis of the residue gave the following percental results: nitrogen, 26.4; carbon, 49.3; hydrogen, 4.6; phosphorus, 0; chlorine (probably as ammonium chloride), 1.3; ash, 0.001 (i. e., a trace). This leaves about 20 per cent oxygen.

From dry powdered gallstones a residue is obtained which is insoluble in alcohol, chloroform, ether and concentrated sulfuric acid and practically insoluble in glacial acetic acid, concentrated hydrochloric acid, concentrated sodium hydroxide and aqueous sodium hydrosulfite. It is dissolved after oxidation with nitric acid or acid potassium permanganate.

This residue is a deeply colored substance. In view of the fact that bile pigments are found in gallstones it might be expected that this insoluble material would consist of very highly condensed ring systems of the pyrrol type, which could be formed from degradation or oxidation products of the bile pigments. This statement is supported by the following facts:

We have (1) the extreme insolubility, (2) the deep color and (3) the results of analysis. None of the methods used gave definite proof that the substance contains condensed pyrrol systems, but the indications are very strong ones.

From the results of analysis one can calculate as an *approximate* empiric formula $(C_4H_5N_2O)_n$

The amount of nitrogen required for pyrrol itself or pyrrol polymers is about 21 per cent. One finds about 27 per cent nitrogen, of which only about 0.05 per cent can be accounted for as ammonium chloride and approximately 1 per cent as hydrolizable amino nitrogen. These corrections still leave one with about 5 per cent more nitrogen than can be accounted for on the basis of a single pyrrol polymer. However, if one accepts some such empiric formula as I have just given as representing a series of pyrrol rings linked together by means of nitrogen and oxygen, the results are reasonable.

It must be stated that I have no direct proof of the presence of pyrrol ring systems and postulate no mechanism for the degradation of bile pigments to give products such as have been suggested; however, no other explanation seems as probable.

SUMMARY

On extracting 700 Gm. of mixed human gallstones with ether, dilute hydrochloric acid, chloroform, alcohol and glacial acetic acid, a dark residue was obtained which corresponded to about 5.6 per cent of the original material and was insoluble in the ordinary solvents. Small amounts of bile acids were present in the alcoholic filtrate. Little or no protein was present in the gallstones. The dark residue was shown to contain practically no inorganic material. It probably consists of polymers containing pyrrol derivatives which are degradation products of the bile pigments and accounts for much of the dark color of some gallstones.

CHANGES IN CARTILAGE AND BONE OF IMMATURE FEMALE GUINEA PIGS DUE TO UNDERNOURISHMENT

WITH CONSIDERATION OF THE PROCESSES OF REPAIR FOLLOWING A PERIOD OF REFEEDING

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AND
RUTH SILBERBERG, M.D.
ST. LOUIS

In the course of our investigations concerning the effect of hormones on cartilage and bone, we noted in guinea pigs given injections of bovine anterior pituitary extract [1] at early periods a temporary loss or lack of gain in body weight; similar observations were made likewise in guinea pigs after administration of thyroid substance [2] as well as after thyroidectomy.[3] The histologic appearances of the skeletal tissues, however, differed greatly under these conditions, and there were no obvious common features which could be attributed to the failure of the body weight to increase.

In order to determine the influence of a loss in weight or of an insufficient gain in weight on the structure of bone and cartilage, we studied the structure of bone and of epiphysial and joint cartilage in the tibias of guinea pigs which had been underfed for a certain length of time in experiments conducted for other purposes by Dr. Leo Loeb.

MATERIAL AND METHODS

The weights of 9 guinea pigs, 1 to 2 weeks old and weighing from 97 to 121 Gm. at the beginning of the observations, were regulated in such a manner as to keep them equal with those of certain test animals. Each time the weight of the underfed guinea pigs tended to rise over that of the test animals, the amount of food was reduced without any other changes in the diet; the amount of food was increased when the weight of the underfed animals was lower than

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These investigations were carried out with the aid of grants from the Committee on Scientific Research of the American Medical Association, from the International Cancer Research Foundation and from the Jane Coffin Childs Memorial Fund for Medical Research.

1. (*a*) Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **32**:1423, 1935. (*b*) Silberberg, M., and Silberberg, R.: Arch. Path. **29**:355, 1940.
2. Silberberg, M., and Silberberg, R.: Growth **2**:327, 1938.
3. Silberberg, M., and Silberberg, R.: Am. J. Path. **16**:505, 1940.

that of the test animals. Thus, the gain in weight was not necessarily completely suppressed during the whole period of observation; the animals were eventually allowed to put on some weight, this gain, however, being limited in accordance with the weights of the test animals. In other words, periods of underfeeding alternated with periods of partial refeeding. The total time of observation, including several periods of underfeeding and refeeding, lasted from twenty-six to forty-nine days. Eight animals of similar age and weight were allowed to gain weight normally and served as controls.

In order to give a more detailed account of the degree of undernourishment and the weights of the various animals, we have prepared the accompanying table, in which the data for each animal are given separately.

Vertical column 4 contains, for each guinea pig, the number of periods of suppression of weight and column 7 the number of periods during which the weight of the animal had been allowed to increase. In columns 5 and 8 the duration of each of these periods is given; column 6 shows the weight which

Data on Undernourishment of Guinea Pigs

Guinea Pig	Initial Weight, Gm.	Period of Observation, Days	Periods of Loss or Standstill of Weight	Duration of Periods of Loss or Standstill of Weight, Days			Weight at Beginning of Periods of Suppression of Gain in Weight, Gm.			Periods of Gain in Weight	Duration of Periods of Gain in Weight, Days		Final Weight, Gm.	Difference of Weight, Percentage of That of Control
				I	II	III	I	II	III		I	II		
649/68	114	29	1	8	..	..	114	...	...	1	21	..	203	+ 2.1
370/40	102	26	1	8	..	..	157	...	...	1	10	..	194	— 2.0
205/34	117	34	2	9	2	..	117	153	...	2	5	18	197	— 6.2
319/38	97	34	2	9	4	..	97	135	...	2	4	17	193	— 8.2
426/10	112	35	1	17	..	..	130	...	...	1	5	..	154	—26.7
230/7	113	49	3	20	4	6	124	145	119	2	3	11	178	—28.9
480/70	106	28	2	15	6	..	106	134	...	1	7	..	125	—30.7
103/8	100	49	3	9	12	6	125	152	170	2	3	3	100	—36.0
433/9	121	49	3	9	22	8	133	145	162	2	2	3	146	—41.6

each animal had at the beginning of each period when the diet was reduced; in column 9 the final weight of each underfed guinea pig is given, and in column 10 the final weights are correlated with the end weights of the normal controls, and the difference between the two groups is expressed in terms of percentage of gain or loss, the weights of the normal controls serving as standards.

In a case in which a period of underfeeding of eight days was followed by a period of adequate feeding lasting twenty-one days the end weight of the animal exceeded that of the normal control by 2.1 per cent. In all other cases the weights of the underfed and temporarily refed animals were lower than those of the normal controls, the difference varying from 2.0 to 41.6 per cent.

MICROSCOPIC OBSERVATIONS

Epiphysial Line.—The width of the zone of endochondral ossification at the upper end of the tibia could be correlated with the degree of underfeeding and with the length of time during which it lasted. The greater the relative lowering of the weight of the animal, the narrower was the epiphysial line. In guinea pigs the end weight of which had

been about 35 per cent below normal and which had been put to death during a period of malnutrition the epiphysial zones were about half as wide as those seen in control animals of corresponding age or weight; then, instead of the normal count of four hypertrophic and ten columnar cells in one cartilage row, only two hypertrophic and six columnar cartilage cells were found. Apparently, in undernutrition the hypertrophic cartilage cells decreased in number more readily than the columnar cartilage cells.

The cartilaginous ground substance showed changes which depended on the duration of the undernourishment rather than on its intensity. At the earlier stages loosening and edematous swelling of the matrix caused separation of its fibrils. Later, the ground substance increased in amount, became collagenous and sclerosed, and in most of the cases in which the process was advanced, took on a preosseous and osseous character. This material protruded in a wedgelike shape from the resting cartilage downward between the cartilage rows and separated them from each other. At the diaphysial side of the growth zone it formed a sclerosed ribbon, staining red with eosin, traversing the zone of hypertrophic cartilage cells and advancing from there upward into the layer of columnar cartilage. The amount of calcium deposited in this area was not only not increased but even less than normal.

The initial changes in the resting cartilage cells, which were seen if the period of underfeeding had not lasted long and if the suppression of gain in weight had not been great, consisted in slight enlargement. After longer periods of undernourishment and with an increasing degree of malnutrition the cells became atrophic, the nuclei small and shrunken, and the cytoplasm narrowed.

The greater the degree and the longer the duration of the underfeeding, the narrower became the nuclei and cystoplasm of the columnar cartilage cells (fig. 1). The narrowing of the epiphysial zone was thus due to atrophy of the cells as well as to decrease in their number. If the malnutrition was pronounced, some cartilage cells could be dissolved. However, in spite of these retrogressive changes, on the whole the columnar cartilage cells revealed a relatively great power of resistance, and their proliferative potencies were maintained to a certain extent, as indicated by the occurrence of mitotic divisions even in cases of the most severe undernourishment.

In very advanced conditions some rows of cartilage cells disappeared, owing to atrophy and solution of cells. Here and there they were replaced by oblong bony plugs extending from the metaphysis into the epiphysis. Under normal conditions, such osseous enclosures are found at the earliest in guinea pigs weighing about 400 Gm. and being 3 to 4 months old, whereas in the underfed animals they could be seen in the

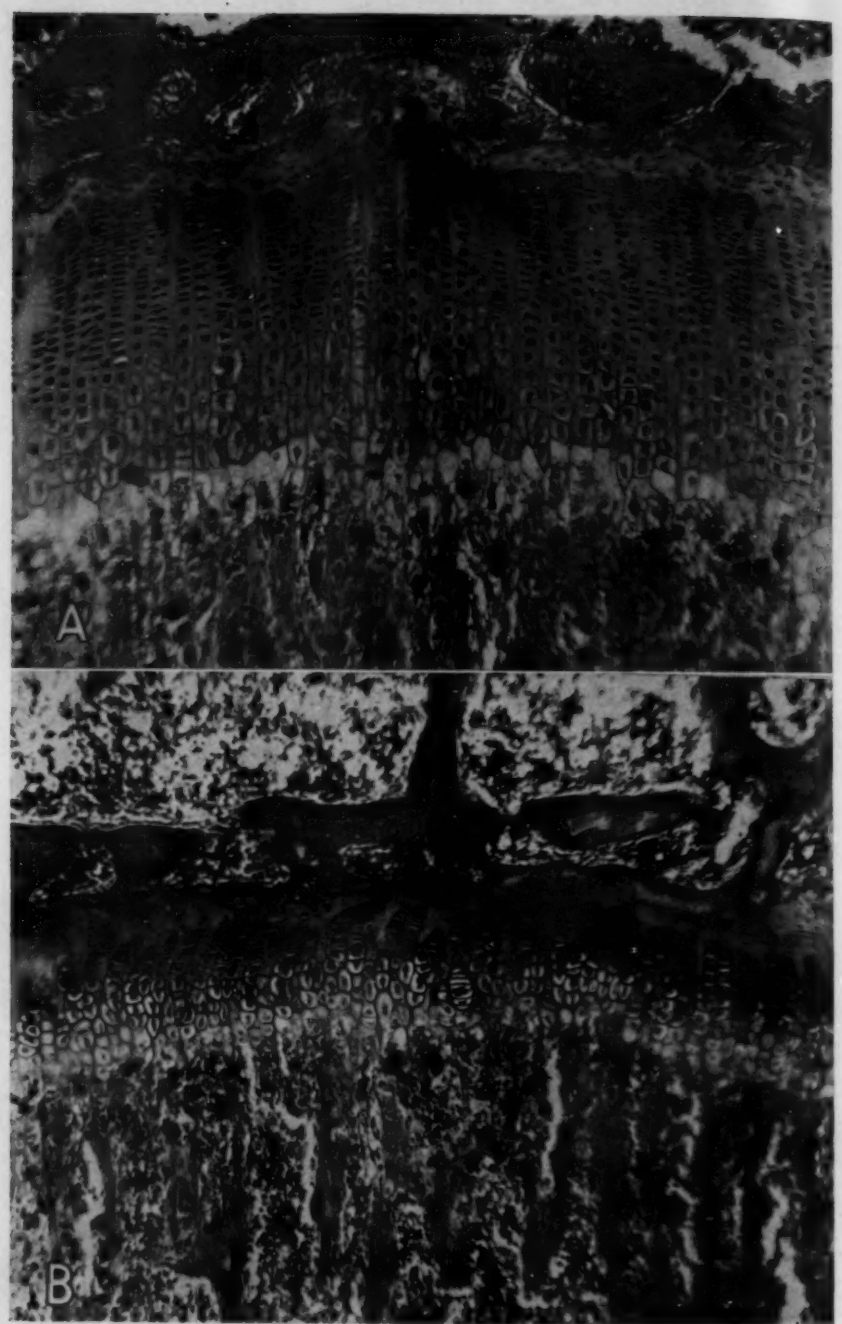

Figure 1

(See legend on opposite page)

2 guinea pigs which had been observed for forty-nine days, which were, at the end of this period, 8 weeks old and had a final weight of 160 and 146 Gm., respectively.

In the hypertrophic cartilage cells shrinkage and decrease in number were observed even earlier than in the columnar cartilage cells. However, with increasing duration of the experiment the retrogressive processes had equally affected both the columnar and the hypertrophic cartilage cells, but the ordinary gradual transition between these two kinds of cells was maintained.

If the examination of the animal had been preceded by a period of refeeding, a reversal of the phenomena described could be noted. The growth zones became gradually wider again in accordance with the length of the period and the degree of refeeding, and the normal number of columnar cartilage cells was restored earlier than the number of the hypertrophic cartilage cells. Thus, in animal 426/10, after five days of refeeding, when the weight was still 26.7 per cent below that of the control animal, the number of columnar cartilage cells in one row had increased again to nine, whereas only two or three hypertrophic cartilage cells were counted. After ten days' refeeding (370/40), when the weight was only 2.0 per cent lower than that of the control, the epiphysial zone was even wider than that of the control guinea pig, and both kinds of cartilage cells were more numerous than ordinarily; there were twelve to thirteen columnar and four to five hypertrophic cells. Subsequent to this period of overbalance, from seventeen days of refeeding on, a gradual return of the number of cartilage cells to the normal value took place, and a normal width of the growth zones was noted.

The sclerosed dense matrix became softer again, and while the cartilage cells proliferated, the amount of ground substance decreased and returned gradually to the normal state.

The resting cartilage cells quickly increased in size, and when the weight of the animals began to equal that of the normal controls the cells assumed again their normal appearance.

EXPLANATION OF FIGURE 1

A, section through the epiphysial disk of the upper end of the tibia of a normal female guinea pig, 8 weeks old, whose weight had increased from 87 to 250 Gm. during the period of observation (forty-nine days). Magnification, × 150.

B, section through the epiphysial disk of the upper end of the tibia of an underfed guinea pig (433/9), 8 weeks old at the time of autopsy, whose weight increased from 121 to 146 Gm. during the period of observation (forty-nine days). The zone of endochondral ossification is distinctly narrowed, the cartilage cells are atrophic, and the cartilage rows are shortened. There is a regular and sharp line of demarcation between the cartilage and the bone marrow. No lines of arrested growth are seen. Same magnification as in *A*.

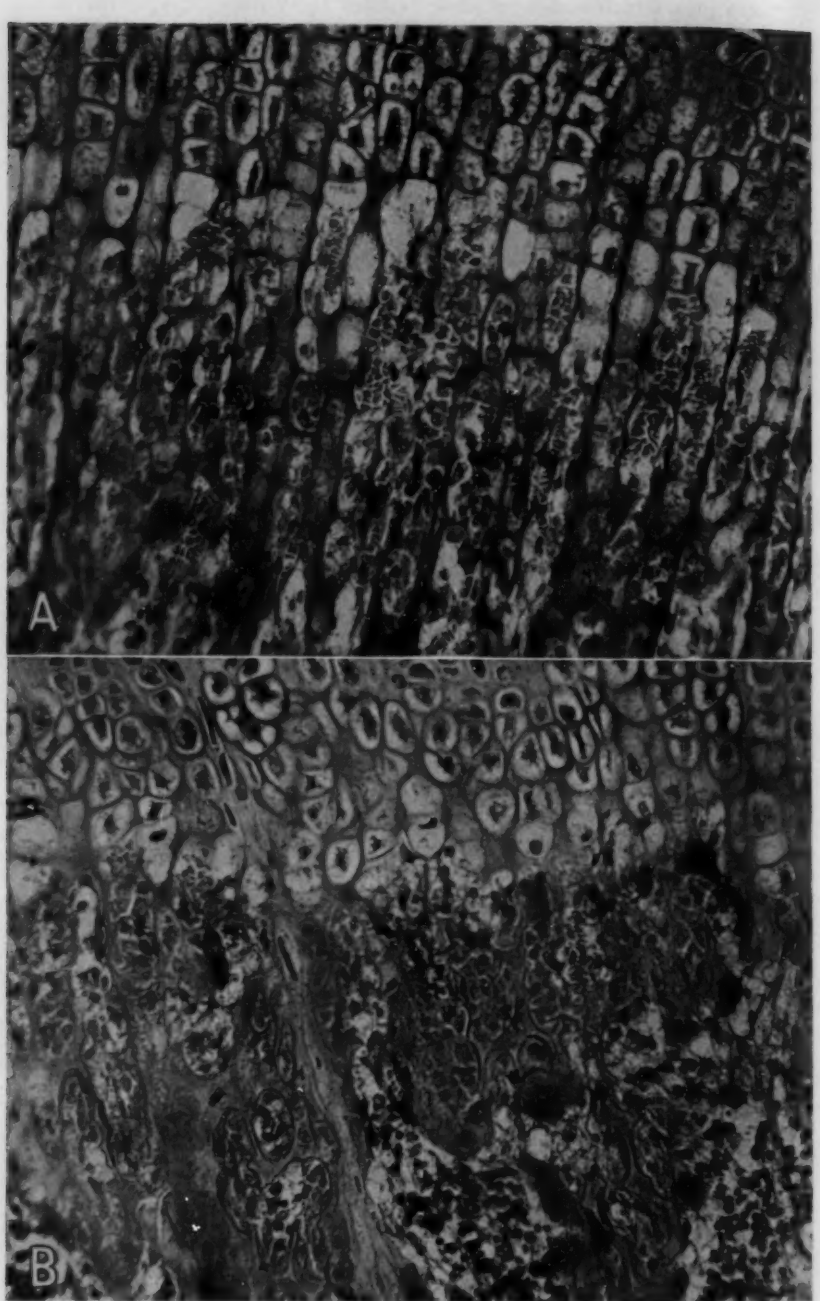

Figure 2

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As early as after three days of refeeding, many columnar cells had regained their normal size and strong mitotic proliferation had set in. During the later stages of restitution the cells became more numerous and larger than in normal guineas pigs of corresponding age and of similar weight. Subsequently further adjustment led to restoration of normal conditions except that the epiphysial cartilage resembled that of normal guinea pigs which were somewhat younger than the control animals.

The hypertrophic cartilage cells, which had undergone atrophy during the periods of malnutrition, increased quickly in size on refeeding, and after five days they had regained their ordinary size; after eight days their normal number of four was restored; later, even slight overproduction of hypertrophic cartilage cells occurred, and as many as five such cells could be present in one row. From seventeen days on, following the beginning of refeeding, a return to the normal count had taken place.

Subepiphysial Zone.—In cases of less severe malnutrition the capillaries approaching the layer of hypertrophic cartilage cells were congested. As compared with the normal (fig. 2 *A*), they were accompanied by a decreased number of osteoblastic epithelioid cells, which here and there proliferated by way of mitosis but less so than ordinarily. Some of these epithelioid cells formed by coalescence multinucleated giant cells. With an increasing degree of undernourishment, the vascularization of the subepiphysial zone became progressively poorer and the zone itself much narrower. Instead of the congested advancing capillaries which are normally found, there was a small strip of connective tissue poor in capillaries, containing epithelioid and large multinucleated cells which presented round, triangular or almost trabecular outlines (fig.

EXPLANATION OF FIGURE 2

A, section through the subepiphysial zone of the guinea pig shown in figure 1 *A*. Magnification, × 220. In the subepiphysial zone, well filled capillaries advance from the bone marrow and corrode the hypertrophic cartilage cells. Beneath the layer of unopened hypertrophic cartilage cells remnants of the corroded cartilage, capillaries and preserved processes of cartilaginous ground substance are seen. Osteoblasts are present, increasing in number toward the metaphysis. Mature thick trabeculae are not seen in this zone.

B, section through the subepiphysial zone of underfed guinea pig 163/8, 8 weeks old at the time of autopsy, whose weight increased from 109 to 160 Gm. during the period of observation (forty-nine days). The zone of advancing capillaries, cartilaginous processes of ground substance and corroded hypertrophic cartilage cells is practically missing. Instead, thick, fairly mature osseous trabeculae, arranged chiefly in a lengthwise direction, adjoin closely, with a comparatively broad base, the unopened hypertrophic cartilage cells, which are smaller than normal. Giant cells are seen. Same magnification as in 2 *A*.

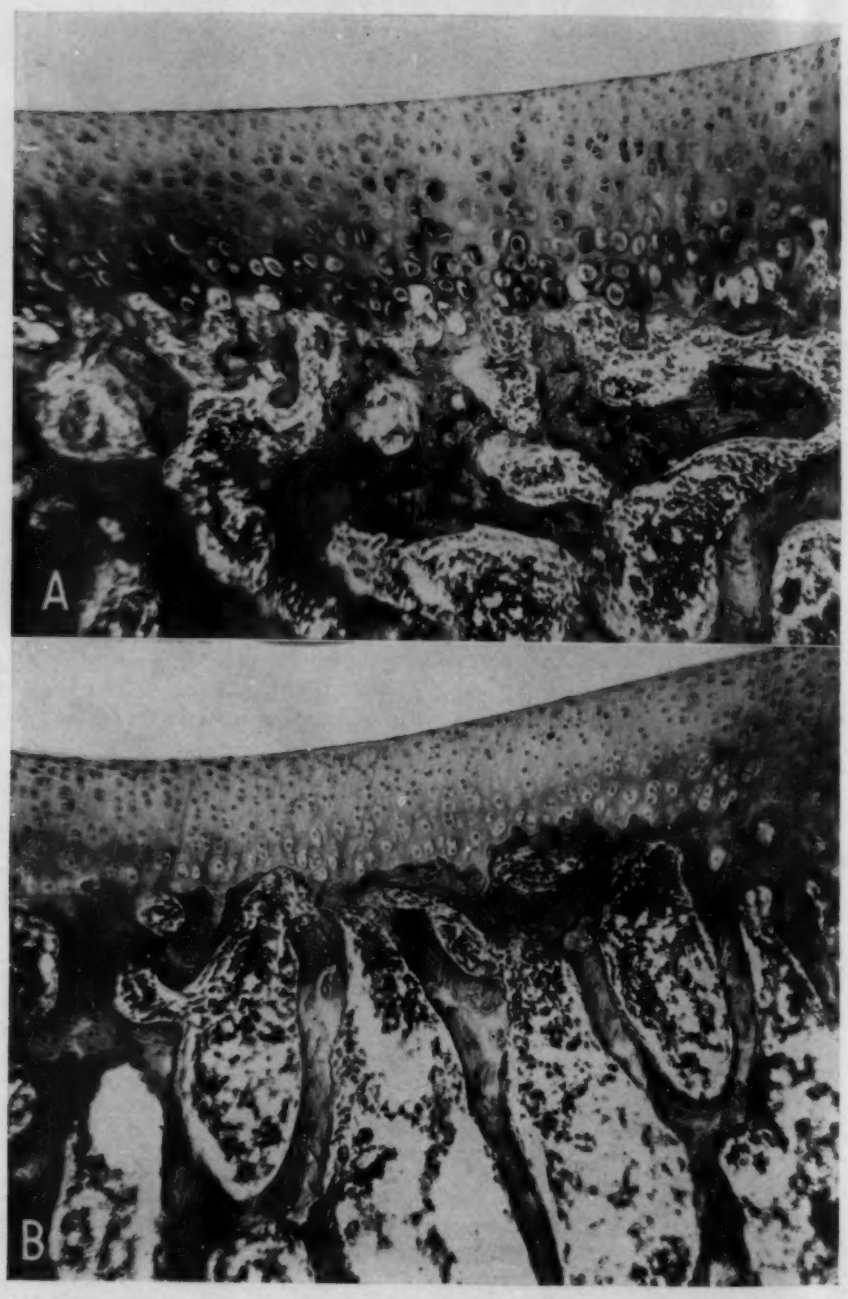

Figure 3

(See legend on opposite page)

2 *B*). These giant cells appeared more numerous than normally. Since, however, they were distributed over a smaller area than under ordinary conditions, there may have been no actual increase in their number. Owing to the narrowing of the entire subepiphysial zone, they also came to lie much closer to the hypertrophic cartilage cells than they do normally. The arrangement of these cells was quite irregular; when a main axis could be recognized, it was oriented either in a lengthwise or in a transverse direction, whereas under normal circumstances the axes extend usually in a lengthwise direction. The cells or cell groups were isolated and always separated from one another by other constituents of the subepiphysial zone, namely, connective tissue, capillaries or hyalinized and calcified ground substance which originally had been cartilaginous. They were never seen to form a continuous layer all across the tibia, nor were there any mature transverse osseous trabeculae which might have been interpreted as so-called lines of arrested growth.

The axes of the bony spiculae were arranged in a longitudinal direction. Because of the narrowing of the subepiphysial zone and the lack of capillaries, the base of the trabecular network was closer to the layer of hypertrophic cartilage and broader than normally. More distally, toward the metaphysis, where the capillaries were more numerous, the trabeculae were narrower than in the proximal parts. The apposition of osteoblastic epithelioid cells around the osseous spiculae was diminished and even rudimentary as compared with the conditions in normal guinea pigs of both similar age and weight. These cells were also smaller than normally. They had lost their round outline and taken on a spindle shape, and their cytoplasm had become narrowed. They did not form a beadlike layer on the surface of the trabeculae as is normal, but they were arranged discontinuously and loosely in an edematous peritrabecular connective tissue. Their tendency

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EXPLANATION OF FIGURE 3

A, section through the surface of the knee joint of the same bone as represented in figure 1 *A*. The various layers of cartilage have a normal arrangement and structure. Note the zone of hypertrophic cartilage cells and the thin bony border lamella between the bone marrow and the hypertrophic cartilage. Magnification, × 150.

B, section through the surface of the knee joint of the same bone as represented in figure 1 *B*. The cartilaginous covering is narrowed; the cells of the various layers of cartilage are atrophic. There are no normal hypertrophic cartilage cells present. The cells of hypertrophic type are smaller than normal hypertrophic cartilage cells. The ground substance in the so-called hypertrophic zone is dense. The osseous border lamella is partly thickened. The trabeculae are thinned out and smooth. There is mucoid atrophy of the bone marrow. Same magnification as in figure 3 *A*.

to coalesce and to form osteoclastic giant cells was likewise decreased. As compared with the normal, the bony spiculae were thinner, smoother and shorter than in guinea pigs of corresponding age; they were, however, more numerous and somewhat thicker and smoother than in normal animals which were younger but which had a similar weight.

On refeeding, the capillaries became rapidly filled again with blood cells, and the subepiphysial zone became wider. An intensified absorption of the subepiphysial bone took place. This process became more marked after from five to seventeen days following the beginning of refeeding. The hypertrophic cartilage cells, the number of which had been restored during this period of gain in weight, were readily broken down by the advancing capillaries, and after seventeen days the process of endochondral ossification did not show any deviation from the normal. Simultaneously, the ameboid epithelioid cells along the trabeculae proliferated strongly by way of mitosis and assumed again their normal tight beadlike arrangement and deposited new bone. Some of them formed giant cells, which acted as osteoclasts. Apposition of bone predominated at the earlier stages of restitution, so that after seventeen days of refeeding more bone had been laid down than under normal circumstances. Later, however, the activity of osteoclasts increased, and thus a normal condition was restored.

Bone Marrow.—The bone marrow of the epiphysis responded more readily to the underfeeding than that of the metaphysis. In those cases, however, in which the loss of weight was as much as 25 per cent or more, the changes in the marrow of the metaphysis and the epiphysis were equally severe. The changes observed were similar to those described in other species (Jackson [4]). The intercellular stroma became swollen and edematous. In this loosened reticular meshwork, especially around congested capillaries, small islands of cellular marrow could be preserved, probably because of relatively good nutritional conditions there. However, over wide areas atrophy and solution had led to destruction of white and red blood cells; karyorrhexis and karyolysis of white cells and shadows of red corpuscles were frequently found. The megakaryocytes and particularly the reticulum cells showed a higher degree of resistance. Numerous phagocytes took into their cell bodies the destroyed material, and their cytoplasm was filled with all kinds of debris and yellowish pigment.

If a gain in weight had taken place following a period of adequate feeding, strong mitotic proliferation of the blood-forming elements led to reappearance of cellular marrow. The repair of the marrow, however, set in at a later stage of refeeding than did that of cartilage and bone, and usually after ten days of refeeding there was still no complete

4. Jackson, C. M.: The Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925.

recovery. However, after seventeen days of refeeding the marrow appeared normal again.

Joint.—The cartilaginous covering of the joint was somewhat thinner than ordinarily. Corresponding to the changes in the epiphysial disk, the hypertrophic cartilage underwent atrophy earlier than did the cells of the pressure, the transitional and the sliding zones. The cells also decreased in number, and the cartilaginous matrix became hyalinized, forming an almost continuous eosinophilic plate in the pressure zone. Ossification of the hypertrophic cartilage had proceeded so far that only the uppermost layer of hypertrophic cells was preserved. A fairly large amount of bone separated the cartilage of the surface from the epiphysial marrow (fig. 3 *B*).

After refeeding, restitution was accomplished by vascular absorption of the osseous lamella, increasing hypertrophy of the cartilage cells and subsequent well balanced replacement of these cells by bone.

Bony Shaft.—The changes in the shaft were comparable to those seen in the osseous trabeculae. With an increasing degree of underfeeding, the epithelioid cells along the inner and outer layers of the compact bone became more and more atrophic. Their proliferative activity decreased, and less bony substance was laid down than under normal conditions. Likewise, osteoclastic giant cells were produced. Thus the cortex appeared fairly smooth and slender. The bone cells were lying in close approximation to one another; their nuclei were round and small, and the cytoplasm was narrow and dense. The haversian canals were narrow. No evidence of either osteomalacic or osteoporotic changes was found.

On refeeding, the haversian canals became wider, the osteocytes of the compact bone increased in size, the connective tissue cells proliferated, and as a result of active formation of osteoblastic epithelioid cells and osteoclastic giant cells a normal balance of apposition and resorption was reestablished.

COMMENT

In growing guinea pigs a lighter degree of underfeeding causes, if any changes at all, only, at later stages, slight atrophy of the euhyaline cartilage of the epiphysial zones. If the undernourishment is intensified to such an extent that the weight of the control animals exceeds that of the experimental animals by 25 per cent and more, the chondromucoid cartilaginous ground substance, after initial swelling, becomes collagenous, thickened and preosseous, and a transverse plate of sclerosed matrix is formed in the zone of the hypertrophying cartilage cells; on the other hand, the processes of calcification are rather decreased. Owing to shrinkage at first of the hypertrophic cartilage cells, subsequently of the columnar and resting cartilage cells decrease in their number, and the

whole epiphysial disk appears reduced in thickness. The proliferative potencies of the cartilage cells are maintained to some extent, but, owing to a decrease in the new formation of cartilage cells, as well as to a decrease in the tendency of these cells to undergo hypertrophy, and the lack of vascularization of the subepiphysial layer, both apposition and resorption of bone are diminished. Thus, in underfed growing guinea pigs less cartilage and bone are found than in normal animals of corresponding age; as compared with control animals of similar weight, such guinea pigs show likewise a decrease in proliferation of the cartilage, but the sclerosis of the matrix is further progressed and the amount of cartilaginous ground substance and of newly deposited bone is increased. The bone marrow is moderately atrophic, even if the degree of underfeeding is so slight that no effect is exerted on the cartilage; in cases of more severe malnutrition, the marrow, especially of the epiphyses, shows severe atrophy and mucoid degeneration.

On refeeding, the changes in cartilage and bone are repaired more quickly than those of the bone marrow, and during the first stages of repair the new formation of cartilage and bone may even exceed the normal. Later, however, the processes of resorption of bone are likewise stimulated, and, therefore, following a period of excessive new formation of tissue, a gradual adjustment leads to a normal condition. This at first accelerated course of repair of the skeletal tissues may furnish an explanation on a histologic basis of the observations of Osborne and Mendel,[5] who reported that subsequent to a period of suppression of growth body growth is accelerated over the normal rate for a certain length of time.

We may then say that undernourishment causes diminution of growth energy rather than serious disorders, an effect comparable to the hypotypical condition of the ovary in the underfed guinea pig (Loeb[6]).

In particular we did not observe any chondrodystrophic changes of the type which have been reported by Diatchenko[7] in the full term fetuses of underfed rabbits but which could not be reproduced in the femurs of undernourished young rats (Silvernale, cited by Jackson[4]). Furthermore, we were unable to detect lines of arrested growth as described by Asada[8] in starved rabbits and by Harris[9] in starved pups, although

5. Osborne, T. B., and Mendel, L. B.: Am. J. Physiol. **40**:16, 1916.

6. Loeb, L.: (*a*) Zentralbl. f. Physiol. **25**:342, 1911; (*b*) Biol. Bull. **33**: 92, 1917; (*c*) Science **45**:591, 1917; (*d*) J. A. M. A. **77**:1646, 1921.

7. Diatchenko, E., in Comptes-rendus du douzième congrès international de médecine, Moscow, 1897, Moscow, 1899, vol. 2, sect. 3, p. 297.

8. Asada, T.: Mitt. a. d. med. Fakult. d. k. Univ. Kyushu Fukuoka **9**:43, 1924.

9. Harris, H. A.: Bone Growth in Health and Disease, London, Oxford University Press, 1933.

our experiments with alternating periods of malnutrition and refeeding should be particularly suited for the demonstration of both these transverse osseous structures and their supposed forerunner, consisting of a strip of closely packed atrophic columnar cartilage cells with intensified calcification of the matrix of the epiphysial zones. The axes of the multinucleated structures in the subepiphysial zone are arranged in a transverse direction more frequently than is normally the case, but they cannot possibly be considered as originating from atrophic cartilage cells or from any cartilage cells at all; they arise from constituents of the bone marrow; moreover, they invariably disappear on refeeding. The same applies in the case of the sclerosed, noncalcified transverse plates of matrix in the zone of hypertrophic cartilage, which likewise disappear readily as soon as the capillary activity in the subepiphysial layer is restored. Our findings in the underfed guinea pigs correspond in this respect to those which we have published previously [1b]; the latter also demonstrate the lack of constancy in the occurrence of so-called lines of arrested growth in guinea pigs after loss or lack of gain in weight subsequent to prolonged administration of bovine anterior pituitary extract, on the one hand, and the presence of transverse bony trabeculae even in normal guinea pigs, on the other.

In former investigations we found that administration of bovine anterior pituitary extract to immature guinea pigs caused narrowing of the growth zones if the ossification of the cartilage, aided by widespread degeneration, predominated over the growth processes, although under this condition both the proliferation of the cartilage and its replacement by bone are relatively and absolutely increased.[10] In contradistinction to these findings in undernourished guinea pigs, the narrowing of the epiphysial disk is not preceded by increased proliferation of cartilage cells, nor is it accompanied by extensive degeneration of the cartilage, but it is due to atrophy of the cartilage, which does not proliferate at the usual rate but, instead, undergoes progressive ossification. Furthermore, in animals which lose weight subsequent to the administration of the anterior pituitary extract, mucoid atrophy of the bone marrow is not found.

On the other hand, we have found that some of the changes observed after underfeeding, namely, the increase in the amount and sclerosis of the cartilaginous matrix and the atrophy of cartilage cells, may also be seen in growing guinea pigs following removal of the thyroid gland.[8] But, while in underfed guinea pigs endochondral ossification proceeds at a diminished rate, in thyroidectomized animals the proliferation of the resting cartilage cells may, on the contrary, be temporarily increased; moreover, the differentiation of columnar into hypertrophic cartilage cells is disturbed, and the ossification of the latter is delayed and irregular.

10. Silberberg, M., and Silberberg, R.: Arch. Path. **26**:1208, 1938.

A temporary loss in weight of young guinea pigs was noted also after feeding of thyroid substance.[2] However, the enlargement of the epiphysial zones, the hyperplasia and hypertrophy of the epiphysial cartilage and the increased absorption of bone which were found after thyroid feeding are the opposite of the changes in cartilage and bone which are observed after underfeeding.

We may then conclude that in young guinea pigs undernutrition exerts a definite depressive effect on the rate of growth of the cartilage but that the changes thus produced will not prevent the typical response of the tissue thus affected to stimulation by certain hormones.

SUMMARY

In growing female guinea pigs underfeeding causes atrophy of the cartilage cells and increased sclerosis of the matrix of the growth zones. The proliferation of the cartilage and its ossification continue, but endochondral and periosteal bone formation proceed at a diminished rate, the degree of diminution depending on the degree of underfeeding.

On refeeding, the sclerosis of the matrix disappears and the cartilage cells reassume their normal appearance. Overproduction of cartilage cells and bone occurs during an early period of refeeding; at later stages normal conditions are restored. No lines of arrested growth or changes which could be interpreted as forerunners of such lines could be observed.

WALLERIAN DEGENERATION IN THE SCIATIC NERVE OF THE RAT

A COMPARATIVE STUDY WITH A SILVER, THE OSMIC ACID AND THE CHLORATE–OSMIC ACID METHODS

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Wallerian degeneration in peripheral nerves was reviewed and studied in detail by Cajal in 1928.[1] In osmic acid preparations of sciatic nerves from rabbits he noted retraction of the myelin at the nodes of Ranvier and accentuation of the Schmidt-Lantermann incisures in the distal stump of the sciatic nerve sixteen hours after the nerve had been sectioned surgically. Four hours later a few of the larger myelin sheaths had fragmented. The earliest degenerative change which he noted in the axis-cylinders in silver-impregnated nerves was a moniliform or varicose appearance, which was seen in some fibers as early as three or four hours after section of the sciatic nerve but usually only after the twelfth hour. Granular degeneration of the axons appeared twenty to thirty hours later. It was shown by other investigators[2] that the Marchi method detected degenerating myelinated fibers much later (in the corticospinal tracts of the cat four days after removal of the cerebral motor cortex).

Because of this long delay before degenerative changes are evident and because of the notable unreliability of preparations stained by the Marchi (also sudan III method), many investigators have resorted to the polarized light technic in an effort to detect the earliest degenerative changes which can be recognized in the myelin sheaths of a sectioned sciatic nerve,[3] in vitamin A and B_1 deficiency and in starvation.[4]

From the Department of Pathology, Harvard Medical School.

This investigation was aided by a grant from the William W. Wellington Memorial Research Fund of Harvard University.

1. Ramón y Cajal, S.: Degeneration and Regeneration of the Nervous System, New York, Oxford University Press, 1928.

2. Swank, R. L., and Davenport, H. A.: Stain Technol. **10**:45, 1935.

3. (*a*) Baldi, F.: Riv. di pat. nerv. **35**:158, 1930. (*b*) Setterfield, H. E., and Sutton, T. S.: Anat. Rec. **61**:397, 1935. (*c*) Setterfield, H. E., and Baird,

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Thus definite evidences of degeneration in the myelin were observed in the distal stump of a rat's sciatic nerve three [3b] and twenty-four [3d] hours after the nerve had been sectioned. These investigators apparently failed to study degenerating peripheral nerves of rats by the more reliable osmic acid or silver methods, as Cajal did, although such control studies seem highly desirable in view of the fact that most of the studies of Cajal and others were done on much larger animals (dogs, cats and rabbits).

The present study is intended to supply the deficiency in data concerning the rate at which degeneration occurs in peripheral nerves of rats as shown by the osmic acid technic for staining myelin and Davenport's silver methods [5] for staining axis-cylinders; to point out that these methods detect degenerative changes in the peripheral nerves of rats far sooner than seems to be appreciated, and to compare the relative ability of the chlorate–osmic acid [6] and conventional Marchi methods to detect degenerating myelin. The mechanism by which the Marchi type of stain (chlorate–osmic acid method) blackens degenerating myelin will be considered also.

METHODS

Growing white rats, weighing approximately 150 Gm. and in apparent good health, were used in the first part of this study. With the rat under light anesthesia induced with pentobarbital sodium, the sciatic nerve on one side was exposed and sectioned as high in the leg as possible, and one or two days later a similar operation was performed on the other sciatic nerve. After one, two, four, eight, twelve, twenty-four, forty-eight, seventy-two and ninety-six hours 2 to 6 rats were killed with chloroform, and the distal stump of each sectioned sciatic nerve was dissected free. Below the knee joint this nerve was tied in situ to a glass rod before it was removed. On removal it was placed directly in a 0.25 per cent osmic acid solution. The remaining nerve proximal to this was fixed for forty-eight hours in neutral solution of 4 per cent formaldehyde gas U. S. P. The formaldehyde-

T. T.: Stain Technol. **11**:41, 1936. (*d*) Prickett, C. O., and Stevens, C.: Am. J. Path. **15**:241, 1939.

4. Sutton, T. S.; Setterfield, H. E., and Krauss, W. E.: Nerve Degeneration Associated with Avitaminosis A in the White Rat, Bulletin 545, Ohio Agricultural Experiment Station, Wooster, Ohio, 1934. Setterfield, H. E., and Sutton, T. S.: J. Nutrition **9**:645, 1935. Lee, J., and Sure, B.: Arch. Path. **24**:430, 1937. Prickett, C. O.; Salmon, W. D., and Schrader, G. A.: Am. J. Path. **15**: 251, 1939.

5. (*a*) Davenport, H. A.: Arch. Neurol. & Psychiat. **24**:690, 1930. (*b*) Davenport, H. A.; McArthur, J., and Bruesch, S. R.: Stain Technol. **14**:21, 1939.

6. Swank, R. L., and Davenport, H. A.: Stain Technol. **10**:87, 1935.

fixed portion of nerve distal to the area of surgical trauma was then divided into four pieces of equal length, and the four pieces were treated, respectively, by the following four technics:

1. The first (also most proximal) segment of nerve was sectioned after freezing and was stained with sudan III.

2. The second segment was stained by the chlorate–osmic acid technic,[6] embedded and sectioned in paraffin, counterstained with 1 per cent aqueous carbol-fuchsin and mounted in xylene-balsam.

3. The third was embedded and sectioned in paraffin and stained with hematoxylin and eosin and by Davenport's silver nitrate technic.[5a]

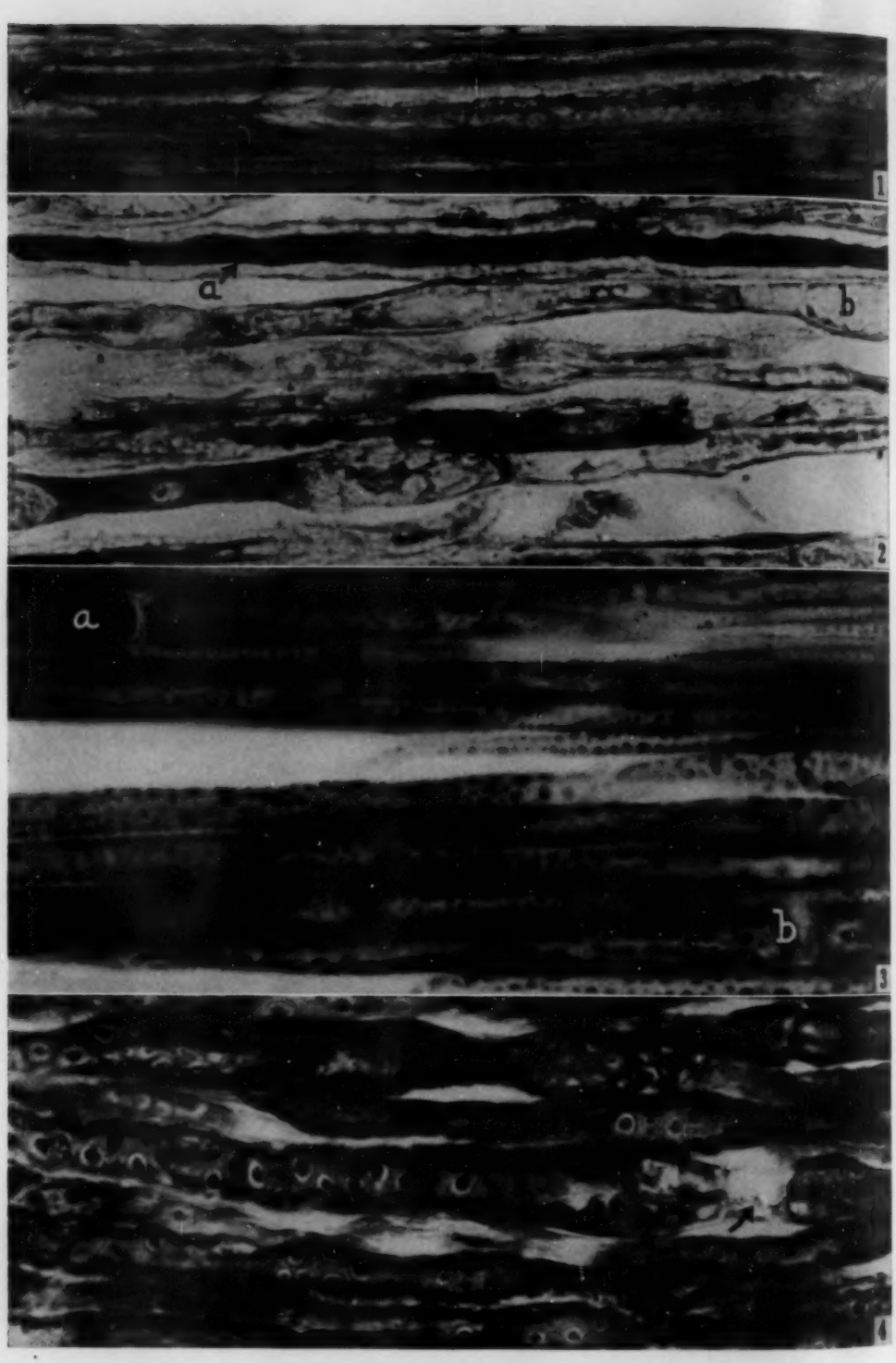
4. The fourth was mordanted in Müller's fluid for two weeks and then stained in Marchi's solution. It was embedded and sectioned in paraffin and mounted in xylene-balsam.

In a group of adult rats, weighing 250 to 300 Gm., one sciatic nerve was divided surgically as described before, and the other used as a control. These rats were subsequently killed with chloroform, and the distal stump of the sectioned nerve and that of the normal control nerve were tied in situ to glass rods and then placed directly in the acidified fixative suggested by Davenport for small nerves. These nerves were then stained by his rapid strong protein silver method.[5b]

Sixty-four rats (110 sciatic nerves) were used in this experiment; of these, 44 (70 nerves) were used to study the very early changes, i. e., those at one, two, three, four, eight, twelve, twenty-four, forty-eight, seventy-two and ninety-six hours. It is with this early group that this paper is concerned.

RESULTS

Degeneration in the Sciatic Nerves of Growing Rats.—Degeneration of the axis-cylinder was most clearly shown by the silver preparations. In the 150 Gm. rats the earliest changes which could be definitely identified as degenerative were observed in an occasional large fiber eight hours after section of the sciatic nerve. At various points these axons were very irregular in contour, swollen, segmented, granular and unevenly stained (fig. 1), and four hours later a few had broken up into small granules. In most of the twenty-four hour preparations many such axons were seen, and in one (fig. 2) the degeneration had progressed so fast that only an occasional intact axon could be recognized. In the forty-eight hour preparations a few fairly normal-appearing axons and others in very early granular stages of degeneration still remained, but in general these granules had scattered or disappeared, and segmentation of the myelin sheath (shown best in osmic acid preparations) had become evident (figs. 7 and 8). The moniliform or varicose-appearing axons, which, according to Cajal, are present in an occasional nerve fiber a few hours after section of the sciatic nerve, were found in normal



Figures 1 to 4

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nerves, so, unless accompanied by more advanced changes, their presence was not used here as a criterion of early degeneration. Swelling of the axon or entire nerve fiber near the node of Ranvier also seemed to occur in the early degenerative period, but this change was difficult to distinguish from that which could be observed in many normal nerves which had been allowed to retract during fixation.

The normal structure and the degenerative changes in the myelin sheaths were seen best in osmic acid preparations. The myelin of a normal nerve (fig. 3) stained by this method appeared to contain many small droplets of black-staining material (lipoidal or myelin droplets) suspended in or surrounded by a gray-staining "supporting structure," the reticular or neurokeratin framework. At the nodes of Ranvier slight swelling of the nerve fibers or slight retraction of the myelin was seen frequently. Swelling of the myelin sheath with retraction at the nodes of Ranvier appeared to occur in more nerve fibers, however, four hours after section of the sciatic nerve, but because the diameter of a nerve fiber depended to a great extent on the degree to which a nerve had been stretched during fixation, little significance could be placed on this observation. The most significant and definite early alterations in the myelin sheaths occurred in and around the lipoidal droplets. In one (of three) four hour preparation and the lipoidal droplets of a few of the larger myelinated sheaths were surrounded by very narrow clear zones or halos. In all eight and twelve hour preparations this change had become more distinct and could be identified in many

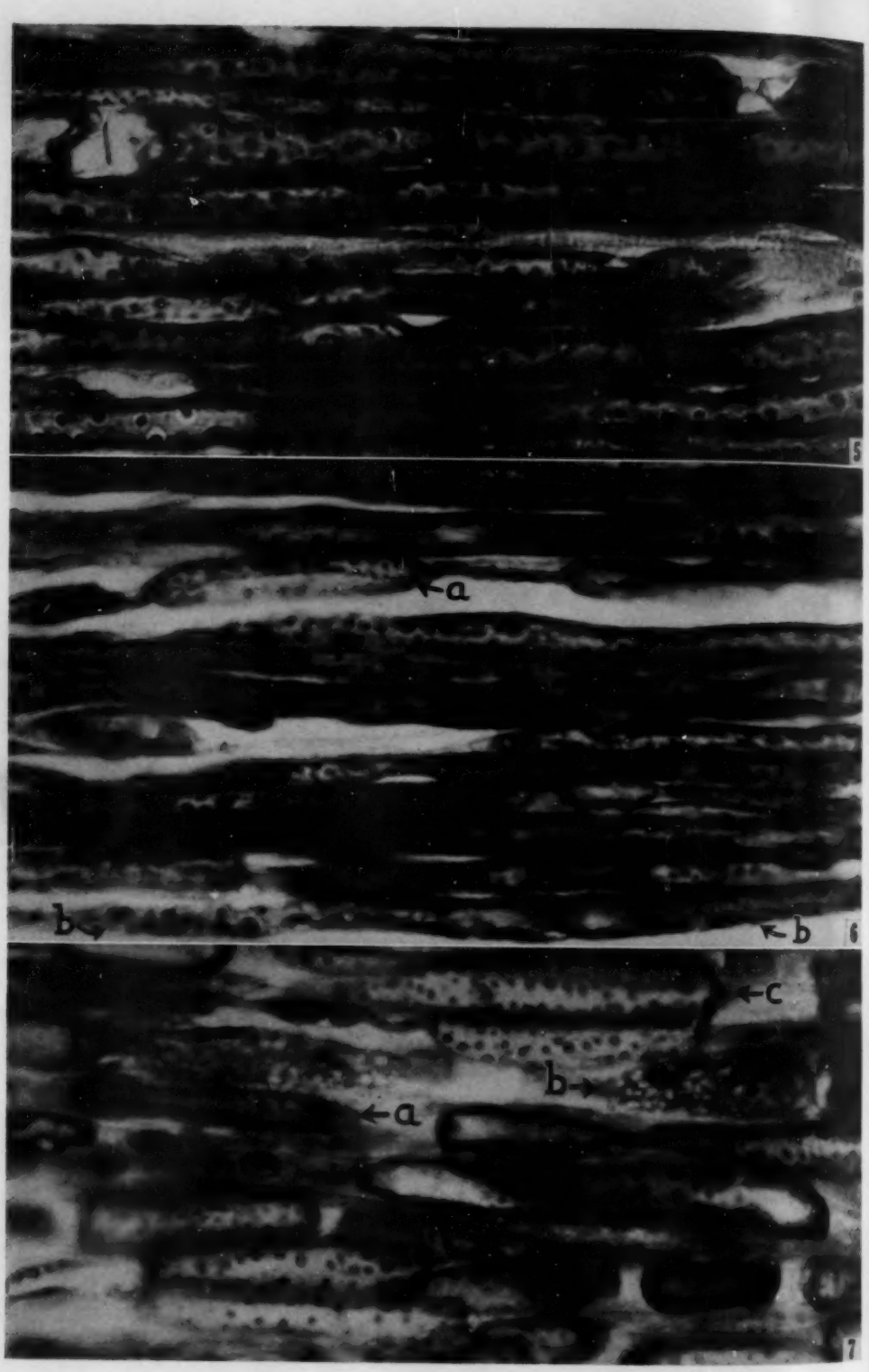
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Silver preparation of the distal stump of a sciatic nerve eight hours after the nerve was sectioned (× 550). Note the irregular, fragmented and unevenly stained axis-cylinder. Swelling of this nerve fiber is also evident.

Fig. 2.—Silver preparation of the distal stump of a sciatic nerve twenty-four hours after the nerve was sectioned (× 550). Note the many axis-cylinders in advanced stages of granular degeneration. At *a* is a normal-appearing axon; in degenerating nerve fibers (*b*) little is left of the axis-cylinder, and segmentation of the myelin is becoming evident.

Fig. 3.—Normal rat's nerve stained by osmic acid (× 550). Note the lipoidal droplets. At *a* the myelin sheath appears slightly swollen in the region of the node of Ranvier, and at *b* slight retraction of the myelin has occurred.

Fig. 4.—Distal stump of a sciatic nerve stained with osmic acid eight hours after the nerve was sectioned surgically (× 550). Note the clear areas or halos around the lipoidal droplets. At the pointer is a definitely pathologic node of Ranvier.



Figures 5 to 7

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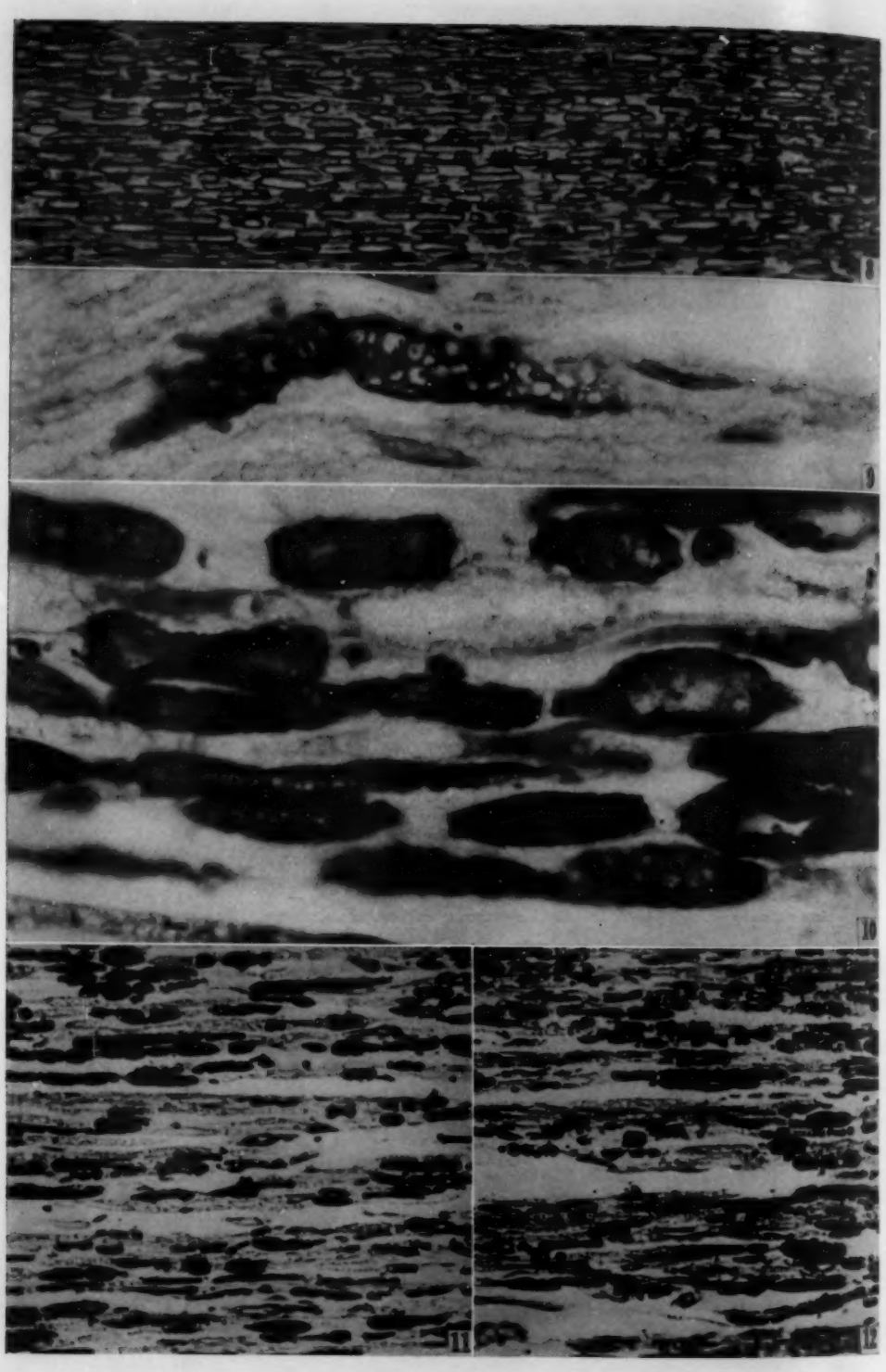
of the medium as well as the large size fibers (figs. 4 and 5). In many of these and other nerve fibers evident swelling of the myelin sheath, definite pathologic retraction and disintegration of the myelin at the nodes of Ranvier and increased prominence of the Schmidt-Lantermann incisures were now evident. In the twenty-four hour preparations (fig. 6) the lipoidal droplets were still present, although smaller and much less discrete and distinct than normal, and the general structure of nearly all of the myelin sheaths was preserved. In a few sheaths the clear zones or halos surrounding the much shrunken lipoidal droplets were discernible, and in a few larger fibers the lipoidal droplets had disappeared entirely, leaving round clear vacuoles. Little further change had occurred at the nodes of Ranvier, and the same could be said of the incisures except that an occasional myelin sheath appeared to be fragmenting at that point. In the forty-eight hour osmic acid preparations (figs. 7 and 8) all nerve fibers had become fragmented into oval myelin globules lying end to end. These segments of myelin varied a great deal in length but usually filled the entire circumference of the sheaths of Schwann. In many such globules the lipoidal droplets could still be seen (fig. 7), although smaller, and at places the surrounding halo was barely visible. In the seventy-two and ninety-six hour preparations the myelin had become broken up into smaller globules, most of which stained uniformly black. In a few places, however, the lipoidal droplets could still be made out. Although initial fragmentation of the myelin appeared to occur frequently at the Schmidt-Lantermann incisures, subsequent globule formation was obviously indiscriminate.

EXPLANATION OF FIGURES 5 TO 7

Fig. 5.—Distal stump of a sciatic nerve stained with osmic acid twelve hours after the nerve was sectioned surgically (× 550). Note the clear areas around the lipoidal droplets. In the upper third of this figure are three nodes of Ranvier away from which swollen myelin sheaths have retracted.

Fig. 6.—Distal stump of a sciatic nerve stained with osmic acid twenty-four hours after the nerve was sectioned surgically (× 550). In fiber *a* segmentation of the myelin is just beginning, and in fiber *b* the halo effect around shrunken lipoidal droplets can be barely identified.

Fig. 7.—Distal stump of a sciatic nerve stained with osmic acid forty-eight hours after the nerve was sectioned surgically (× 550). All nerve fibers have become segmented. The lipoidal droplets are less distinct. At *a* they are markedly shrunken and appear inside clear vacuoles; at *b* most of them have vanished leaving clear vacuoles, and at *c* many fairly normal-appearing lipoidal droplets can be seen indistinctly inside a segmented myelin sheath.



Figures 8 to 12

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The chlorate–osmic acid method did not detect degeneration as quickly as did the osmic acid and silver methods, but it was more sensitive in this respect than the Marchi method. Twenty-four hours after the sciatic nerve had been sectioned a few large myelin sheaths stained black in the chlorate–osmic acid solution, and twenty-four hours later a considerable majority of these sheaths could be identified in this manner (fig. 11). The first staining occurred at the periphery of the small round spaces occupied by the lipoidal droplets, and later the entire reticular framework became uniformly gray and finally black in that portion of the nerve (fig. 9). The lipoidal droplets remained colorless throughout their existence except in a few places where they appeared as very small irregular black masses inside relatively large clear vacuoles (figs. 9 and 10). With continued fragmentation of the myelin sheath and disappearance of the lipoidal droplets, the myelin sheaths became uniformly black and ultimately appeared much the same as the osmic acid preparations (figs. 8, 10, 11 and 12).

A few black globules had just appeared in the conventional Marchi preparations, and but a few droplets of fat were stained red by the sudan III technic in the four day preparations, just three days later than staining was observed in the chlorate–osmic acid preparations.

Degeneration in the Sciatic Nerves of Adult Rats.—The sciatic nerves of the adult rats were studied in silver preparations only.[5b] The granular stage of degeneration became evident in these animals

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EXPLANATION OF FIGURES 8 TO 12

Fig. 8.—Distal stump of a sciatic nerve stained with osmic acid forty-eight hours after the nerve was surgically sectioned (× 166). All myelin sheaths are broken up into numerous segments or globules.

Fig. 9.—Distal stump of a sciatic nerve stained by the chlorate–osmic acid method twenty-four hours after the nerve was sectioned surgically (× 550). In the single stained myelin sheath note the uniform gray or black staining of the neurokeratin, whereas the lipoidal droplets remain unstained or blacken only in part.

Fig. 10.—Distal stump of a sciatic nerve stained by the chlorate-osmic acid method forty-eight hours after the nerve was sectioned surgically (× 550). Note that the lipoidal droplets remain unstained and appear as clear vacuoles in many of the globules of myelin.

Fig. 11.—Distal stump of a sciatic nerve stained by the chlorate–osmic acid method forty-eight hours after the nerve was sectioned surgically (× 166). Note the large percentage of fibers that are already staining.

Fig. 12.—Distal stump of a sciatic nerve stained by the chlorate–osmic acid method ninety-six hours after the nerve was sectioned surgically (× 166). Note further increased fragmentation and staining of myelin.

no sooner than twenty-four hours after section of the sciatic nerve, and then in only a few of the larger fibers. Without pursuing this aspect of the problem further, it was evident that degenerative changes in the axis-cylinders could be identified sooner in sciatic nerves from growing 150 Gm. rats than from older 300 Gm. rats.

COMMENT

In young growing rats, definite degenerative changes were observed in the axis-cylinders and myelin sheaths of the peripheral stump of the sciatic nerve eight hours after it had been sectioned, and in 1 rat the myelin sheath alone was found abnormal four hours earlier. Although degenerative changes could not be seen in the axis-cylinder earlier than this, it is not doubted that the process of degeneration was initiated there. Similar early changes were demonstrated in the axis-cylinders and the myelin sheaths of rats by the polarized light technic,[3b] and they corresponded fairly closely in time to those described in this paper.

Granular degeneration of the axis-cylinder appeared in adult rats approximately twelve hours later than it did in much younger ones, and in larger animals similar changes did not occur until much later—in the rabbit thirty to forty hours after section of the sciatic nerve[1] and in the dog forty to fifty hours later.[7] With regard to the rabbit, fragmentation of an occasional myelin sheath was observed in sixteen and twenty hour preparations by Cajal, although similar changes, according to Ranson, appeared in the dog after the axis-cylinder had undergone definite granular degeneration.

By means of the polarized light technic, Setterfield and Sutton[3b] observed that marked swelling of the axis-cylinder and myelin sheath could be seen three hours after the sciatic nerve of a rat had been sectioned. The observation that the diameter of an axis-cylinder or myelin sheath was determined largely by the extent to which a nerve was stretched during fixation suggests that this swelling was due to retraction of the nerves which he studied. Because of the marked normal variation which is possible from this cause, swelling of a nerve fiber per se has not been used here as a criterion of degeneration.

Many of the early changes which occur in the myelin sheath of a degenerating sciatic nerve of the rat are concerned primarily with the lipoidal droplets. First clear areas or halos were noticed around these droplets. Subsequently they diminished in size and finally disappeared from some sheaths. Concomitant with this change and apparently irrespective of segmentation of the nerve fiber, more and more myelin sheaths stained black by the chlorate–osmic acid method.

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7. Ranson, S. W.: J. Comp. Neurol. **22**:487, 1912.

Setterfield and Sutton [3b] observed that finely granular masses of isotropic material appeared in the myelin sheaths (which are normally anisotropic) three to six hours after the sciatic nerve of a rat had been sectioned, and that these gradually increased in amount until large isotropic globules were present. This observation plus those discussed in the immediately preceding paragraph suggests that this isotropic material in a degenerating nerve has its origin in the myelin droplets, which normally are anisotropic. These become changed, during degeneration of a nerve, and the products which are liberated migrate or become transferred into the neurokeratin. Because of their presence, the neurokeratin stains black by the chlorate–osmic acid method, whereas the lipoidal droplets remain unstained to a large extent. This hypothesis does not explain why the neurokeratin of a degenerating nerve fiber does not stain black by osmic acid alone.

This hypothesis is supported by separate observations that will be recorded in another paper.[8] The lipoidal droplets in the sciatic nerves of starved rats became smaller and in a few instances disappeared entirely from some myelin sheaths. During this apparent removal of the myelin droplets, many of the myelin sheaths stained black in the chlorate–osmic acid stain. Except that other morphologic evidences of degeneration (granular degeneration of axis-cylinders and fragmentation of myelin sheaths) were lacking, the black-staining myelin sheaths in these preparations were identical with many of those observed in degenerating sciatic nerves.

It appears quite obvious that the chlorate–osmic acid method (also the Marchi method) does not specifically stain degenerating nerve fibers. Blackening of myelin sheaths by either method indicates that an apparent change of the lipoidal droplets is occurring. This may be due to wallerian degeneration of that fiber or to starvation alone. In the latter condition the structure of the myelin sheath and axis-cylinder remains intact, whereas in the former disintegration of the axon and myelin sheath occurs. Because of its greater sensitivity, the chlorate–osmic acid stain will probably blacken more myelin sheaths in starved rats than will the conventional Marchi method, but as it reveals the structure of a nerve quite clearly, intact nerve fibers can be recognized.

When the Marchi technic is used to trace experimentally induced degeneration in the nervous system of the cat or dog, fourteen days after the trophic cell bodies have been destroyed is usually considered time enough for degeneration to occur. In smaller animals, i.e., rabbits, eight to ten days is probably optimum. Because of its greater sensitivity, it is not necessary to wait so long when the chlorate–osmic acid method

8. Observations made in collaboration with Dr. S. B. Wolbach and Dr. O. A. Bessey.

is used. Six days in the rat, eight in the rabbit and ten in the cat are sufficient for a maximum number of degenerating myelin sheaths to become stainable. It is extremely unlikely that confusion due to starvation would arise when these degeneration times are used, for even rats must starve for at least three weeks before their myelin sheaths exhibit the effect of starvation.[8]

It is outside the scope of this paper to discuss whether the lipoidal droplets which were observed in osmic acid preparations are present in living nerves as such or are produced by fixation. It is sufficient to point out here that they were altered consistently during degeneration and starvation.[8] It should be pointed out, however, that extreme care must be exercised if good osmic acid preparations are to be obtained. If a nerve is stretched taut during fixation, the myelin droplets will not be discernible, and if it is too loosely supported while it is in the osmic acid solution, the nerve fibers will vary abnormally in size and the myelin droplets be poorly defined.

SUMMARY

Wallerian degeneration was studied in growing and adult rats, using the osmic acid, Davenport's silver and the chlorate–osmic acid methods. In young rats (150 Gm.) the earliest reliable evidences of degeneration appeared in axis-cylinders of the distal stump of the sciatic nerve, approximately eight hours after the nerve had been sectioned. In adult rats (300 Gm.) similar degenerative changes appeared in the axis-cylinders approximately twelve hours later. At about the same time changes were observed in the myelin involving an apparent transfer of lipoids from the myelin droplets probably to the neurokeratin. During this transfer the neurokeratin stained black in the chlorate–osmic acid solution. About twenty-four hours after section of the sciatic nerve a few degenerating myelin sheaths stained black by the chlorate–osmic acid method, and twenty-four hours later a majority of the sheaths stained. A few black globules were found in the conventional Marchi preparations ninety-six hours after section of the sciatic nerve.

INFLUENCE OF SULFANILAMIDE AND SULFAPYRIDINE ON EXPERIMENTAL PNEUMOCOCCIC PNEUMONIA IN RATS

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AND
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The role played by the cellular defenses of the host in bacterial infections treated with sulfanilamide or sulfapyridine (2-[paraaminobenzene sulfonamido]-pyridine) is still not understood. An approach to the problem was sought through the medium of experimental pneumococcic (type III) pneumonia in the rat, in which a well defined inflammatory pattern displaying uniform evolution could be expected; an investigation was made of the influence of sulfanilamide and sulfapyridine on the extent and course of the induced inflammatory process, the character and cell type of its exudate, and the extent of phagocytosis.

The genesis and the histologic nature of this type of murine pneumonia were first described by Gunn and Nungester.[1] By using a slight modification of their technic, it was possible to produce pneumonias which were uniform in clinical and pathologic aspects and which bore a striking gross and histologic resemblance to the disease in man.

TECHNIC

In the course of these experiments 119 young albino rats, free from respiratory disease and weighing from 175 to 250 Gm., were subjected to surgical exposure of the trachea under ether anesthesia. With a tuberculin syringe, 0.3 cc. of a mixture of 4 per cent gastric mucin and type III pneumococci (strain H3)[2] was injected into the exposed trachea; the inoculum was prepared by diluting an eighteen hour blood broth growth of pneumococci tenfold in mucin. Animals in the treated groups were given sulfanilamide in olive oil emulsion in daily doses of 0.75 mg. per gram of rat by the subcutaneous route. Sulfapyridine was given orally in a 20 per cent acacia medium by means of a metal catheter attached to a tuberculin syringe. The dosage was the same as in the experiments with sulfanilamide. The first administration of the drug was made within two hours after the operation and repeated once daily. Except for animals in survival experiments, the rats were divided into groups and killed serially at daily intervals. Just prior to killing them, tail blood was obtained for culture. Autopsies were performed at once under sterile conditions, and cultures of the lungs were made.

From the Departments of Pathology and Medicine, New York University College of Medicine.

1. Gunn, F. D., and Nungester, W. J.: Arch. Path. **21**:813, 1936.

2. This strain of pneumococci was isolated from the blood of a patient with type III pneumonia and passed through mice every two days for the four weeks intervening between its isolation and its use in the experiment.

In three experiments there was deviation from the technic just described. In one, 3 animals were subjected to the standard operative procedure but were given mucin alone. In the second, 4 animals were given additional sulfanilamide seventeen hours prior to the introduction of pneumococci and were put to death one day after infection. In the third, the effect of delayed treatment on survival was observed by treating 7 rats for the first time after thirty hours of infection, a period of time determined by the first death of a control.

Survival experiments were designed to demonstrate the efficacy of each compound in preventing death from pneumonia. Ten animals were treated with sulfanilamide at daily intervals for eight days, and those which survived fifteen days were then put to death. A similar number of rats served as controls; the whole study was performed in two experiments several weeks apart, each experiment comprising 10 animals, evenly divided into treated and a control group.

The same procedure was employed in studying the ability of sulfapyridine to prevent death except that treatment was carried through the fifteenth day in an effort to produce sulfapyridine renal calculi. This survival test was done in a single experiment with 5 control and 5 treated rats.

The typical experiment comprised 15 to 20 rats. Five of these served as controls, and their death from lobar pneumonia on or before the fifth day was required as an index of maximum virulence of the pneumococcus strain. Animals treated with sulfanilamide were killed in groups of 3 or 4 at daily intervals through the seventh day. In the sulfapyridine experiments rats were put to death on the first and second days only. Eighty-nine animals were employed in the study of the first compound; thirty in the study of the latter. Control rats for the one day period were put to death, whereas control material for the second and fourth days was made up for the most part of animals dying on these days. The observations on control rats which died and in those which were put to death at the same time were essentially similar.

The virulence of the pneumococcus strain was maintained by continuous rat passage via lung cultures except during the summer months, when the culture was preserved by lyophilizing. It was found that lyophilized cultures possessed the capacity, without alteration, to produce lethal pneumonia for as long as eight months, after which there appeared to be partial loss of virulence.

The dosage used was an overwhelming one, and it was felt that no purpose would be served in establishing the minimum lethal dose in rats. Strain H3 was lethal to mice in dilutions of 10^{-7}. The first three strains that were tested, though highly virulent for mice (10^{-8}), failed to produce pneumonia in rats. This discrepancy between the virulence for mice and that for rats has been pointed out by others[3] and makes standardization based on virulence for mice untrustworthy in regard to infections of rats.

Blocks of lung (all lobes), liver, spleen, kidney and bone marrow were sectioned for microscopic study. The hematoxylin-eosin stain, Mallory's phosphotungstic acid–hematoxylin stain and a combined Giemsa-Wright stain,[3a] the latter for bacteria, were employed. In selected instances frozen sections were prepared for lipid stains.

OBSERVATIONS IN EXPERIMENT WITH SULFANILAMIDE

1. *Effect on Survival Time (Fig. 1).*—The death rate among 26 controls was 96 per cent, and the average lapse of time before death was

3. Gross, P.: Personal communication to the authors.

3a. The following combination of the Giemsa and the Wright stain was found effective for the demonstration of bacteria: After removing paraffin in the

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three and two-tenths days, with a range of one to seven days. With the exception of 2 rats that died late (fifth and seventh days) all controls died on or before the fourth day.

Of ten animals treated eight days and designated for death on the fifteenth day, 1 died on the eighth and 1 on the eleventh day. Among the 37 treated animals serially put to death (one to seven days) there was a single spontaneous death, and that occurred on the fifth day.

2. *Evolution of Pneumonia in the Controls During the First Twenty-Four Hours After Infection (Table 1).*—Six controls killed at twenty-

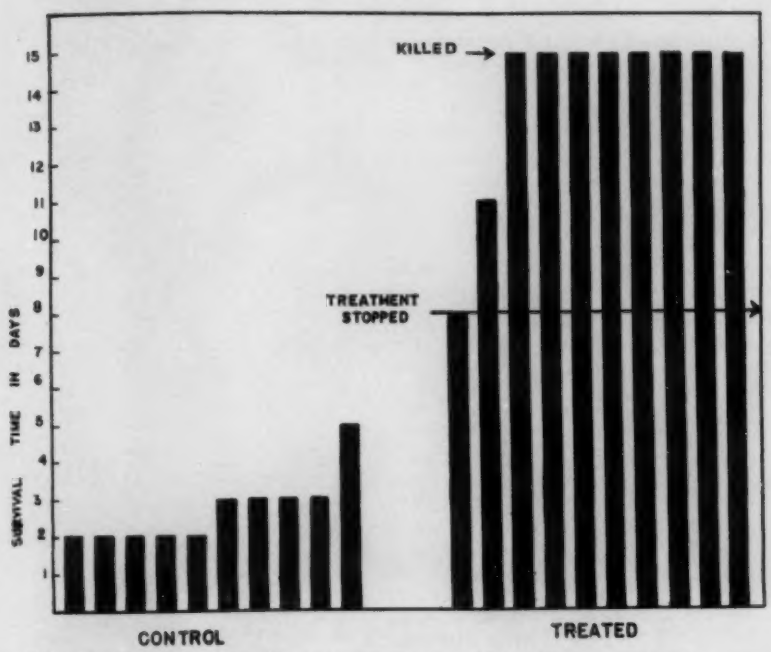

Fig. 1.—Effect of sulfanilamide on survival. Each column represents 1 rat. Each rat received 0.3 cc. of a 1:10 dilution of a culture of type III pneumococci in mucin by the transtracheal route. Each treated rat received 0.75 mg. of sulfanilamide per gram of body weight daily through the eighth day.

four hours uniformly exhibited diffuse fibrinopurulent pneumonia, almost lobar in extent, involving on the average two and one-half lobes. (The upper and lower lobes of the right lung and the upper lobe of the left lung were involved with similar frequency.) The lesion, easily discernible, always involved the hilar aspect of the lung, whence it appeared to

usual manner (1) stain with Giemsa solution overnight (1 drop to 1 cc. of distilled water); (2) rinse in distilled water; (3) immerse in Wright's stain for ten to fifteen minutes; (4) differentiate in 80 per cent alcohol under microscopic observation; (5) dip quickly in 95 per cent alcohol and transfer to absolute alcohol for two to three minutes; (6) clear in xylene and mount in balsam.

TABLE 1.—*A Composite of the Pathologic and Bacteriologic Observations on Serially Killed Sulfanilamide-Treated and Control Rats*

Experiment	Rats	Extent of Pneumonia ‡	Microscopic Change	Pleural Involvement	Positive Cultures		Microscopic Estimate of Bacteria
					Lung	Blood †	
Controls killed after 1 day	6	1 + 1 ++ 4 +++	Diffuse fibrinopurulent exudate	6+	6	2 (4)	++++
Treated rats killed after 1 day	12	4 + 3 ++ 5 none	3 with patchy lobular exudate 6 with diffuse fibrinopurulent exudate 3 with patchy mononuclear exudate	1+	5	0	2 + 2 ++ 8 none
Controls that died on 2d day	11	1 + 4 ++ 4 +++ 2 ++++	Diffuse fibrinopurulent exudate	9+	11	8 (8)	++++
Treated rats killed on 2d day	8	2 + 3 ++ 3 none	4 with diffuse fibrinopurulent and mononuclear exudate 4 with patchy mononuclear exudate	2+**	3	0	3 + 5 none
Controls that died on 4th day	9	5 ++ 3 +++ 1 ++++	Diffuse fibrinopurulent exudate	9+	9	3 (3)	++++
Treated rats killed on 4th day	9	2 + 2 ++ 1 +++ 4 none	5 with diffuse fibrinopurulent exudate (4 resolving) 4 with focal mononuclear exudate	3+**	2	0	3 + 1 ++ 1 ++++ 4 none
Treated rats killed on 5th day	3	3 +	2 with diffuse fibrinopurulent exudate (both resolving) 1 with focal mononuclear exudate	1+*	1	0 (2)	1 + 1 ++++ 1 none
Treated rats killed on 6th day	4	1 + 2 ++ 2 none	2 with diffuse fibrinopurulent exudate (both resolving) 2 with focal mononuclear exudate	1+*	1	0 (2)	2 + 2 none
Treated rats killed on 7th day	3	1 ++ 2 none	1 with diffuse fibrinopurulent exudate (resolving) 1 with focal mononuclear exudate 1 with interstitial pneumonia	1+*	1	0	1 + 2 none
Treated rats killed on 15th day	8	1 + 1 ++ 6 none	2 with diffuse fibrinopurulent exudate (1 resolving) (1 organizing) 4 with focal mononuclear exudate 2 normal	3+***	0	0	0

* Each asterisk represents an instance of pleural involvement in which the mononuclear form was the predominant cell type; in all other instances pleural inflammation was purulent.
† Figures in parentheses designate the number of animals whose blood was cultured.
‡ Each + represents involvement of about one-half lobe.

take origin, for at times this was the only area involved. Spread was always posterior at first, where at twenty-four hours there could be seen a zone of grey-red airless solid lung with deep red, slightly crepitant tissue at its periphery. The pleura was normal mascroscopically in all but 1 animal; in this animal there was an empyema.

On microscopic study (fig. 3 *A*) the gray-red hilar zone was seen to be an area in which the alveoli were moderately filled with an exudate of well preserved polymorphonuclear leukocytes, abundant edema fluid and fairly coarse fibrin nets (fig. 3 *B*). Here pneumococci were present in numbers too great to count (fig. 3 *C*). These were for the most part

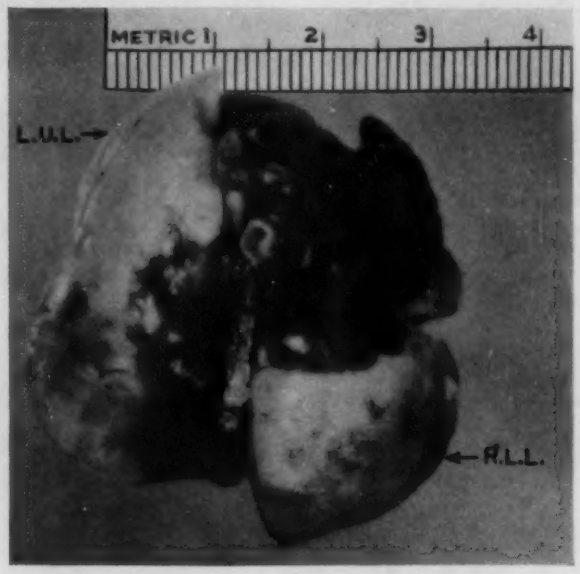

Fig. 2.—A posterior view of the lungs of an untreated rat which died on the fourth day, showing consolidation of the lower lobe of the right lung and of the upper lobe of the left lung. The other lobes are markedly congested.

extracellular collections of diplococci, free in the alveoli, although a small number of phagocytosed organisms were observed.

The lumens of all divisions of the bronchial tree were densely packed with polymorphonuclear leukocytes, and the lining mucous membrane was fractured, desquamated or entirely absent. Purulent septal cellulitis and periarteritis were fairly conspicuous (fig. 3 *A*).

Moving out from the hilus (corresponding to the zone of red described) one found a more recent lesion. Microscopically this comprised edema fluid, leukocytes sparsely filling the alveoli and occasional erythrocytes. In this zone the engorgement of capillaries was intense. Regularly, edema fluid without cells but rich in bacteria spread out beyond this.

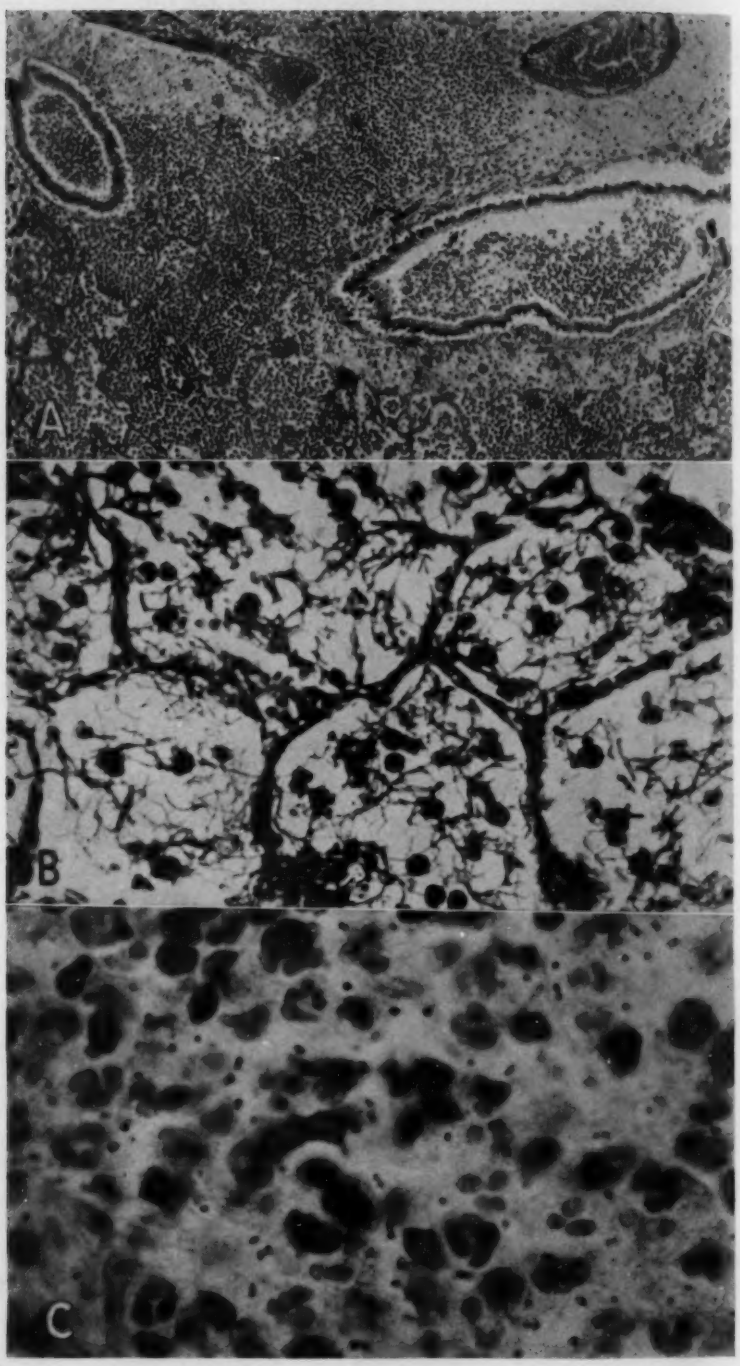

Figure 3

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In all of the twenty-four hour controls there was microscopic pleural inflammation, characterized by the presence of fibrin, polymorphonuclear leukocytes and monocytes.

3. *Pathologic Observations in Controls at Two and Four Days After Infection.*—Eleven controls were studied at two days, and all showed extensive lobar consolidation with total involvement of more than half the pulmonary parenchyma. The gross appearance was similar to the one day consolidation except in two respects. Empyema fluid covered the visceral pleura in most instances (7 of 11), and the coloring of the involved lung tended to be more uniform throughout; the consolidated areas now assumed a dirty, dark red appearance.

Histologically, this stage was characterized by a more solid leukocytic exudate in which fibrin was most abundant and dense. In 5 animals abscesses were present; cultures of the lungs yielded pure growths of pneumococci, thus excluding secondary invaders as etiologic agents in the formation of these abscesses while evidence was lacking to support a vascular basis for their presence. However, bright red infarcts, visible under low magnification, were seen in 2 other animals, and the adjacent pulmonary vessels exhibited homogeneous platelet thrombi.

Estimation of the bacteria present indicated myriads of diplococci free in the alveoli. Intracellular forms were found in most oil immersion fields, but they were a distinct minority of the total bacterial flora.

Four controls died on the third day. The changes in these were not significantly different from those previously described.

The death of 9 untreated rats on the fourth day provided the control material for this period. The gross findings in these animals differed little from what was observed in controls at two days. There was macroscopic pleural involvement in all, and in 8 rats frank empyema was noted. The consolidated areas had become gray-white (fig. 2), markedly increased in size and solid; when not involving a whole lobe, they dwarfed and compressed the air-containing lung tissue.

Microscopic examination revealed fibrinopurulent pneumonia, with a dense leukocytic exudate packing the alveoli. There was much degen-

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EXPLANATION OF FIGURE 3

A, photomicrograph of a section of a lung of a control rat that was put to death at twenty-four hours, showing a dense fibrinopurulent exudate, abundant edema fluid, interstitial inflammation and purulent endobronchitis; hematoxylin and eosin; × 75.

B, the same as *A* but stained with Mallory's phosphotungstic acid–hematoxylin to show the character of the fibrin nets; × 300.

C, the same as *A* but stained with the Wright-Giemsa method to show the extracellular distribution of the pneumococci; under oil; × 900. The refractory zone seen around several of the pneumococci suggests the presence of capsules.

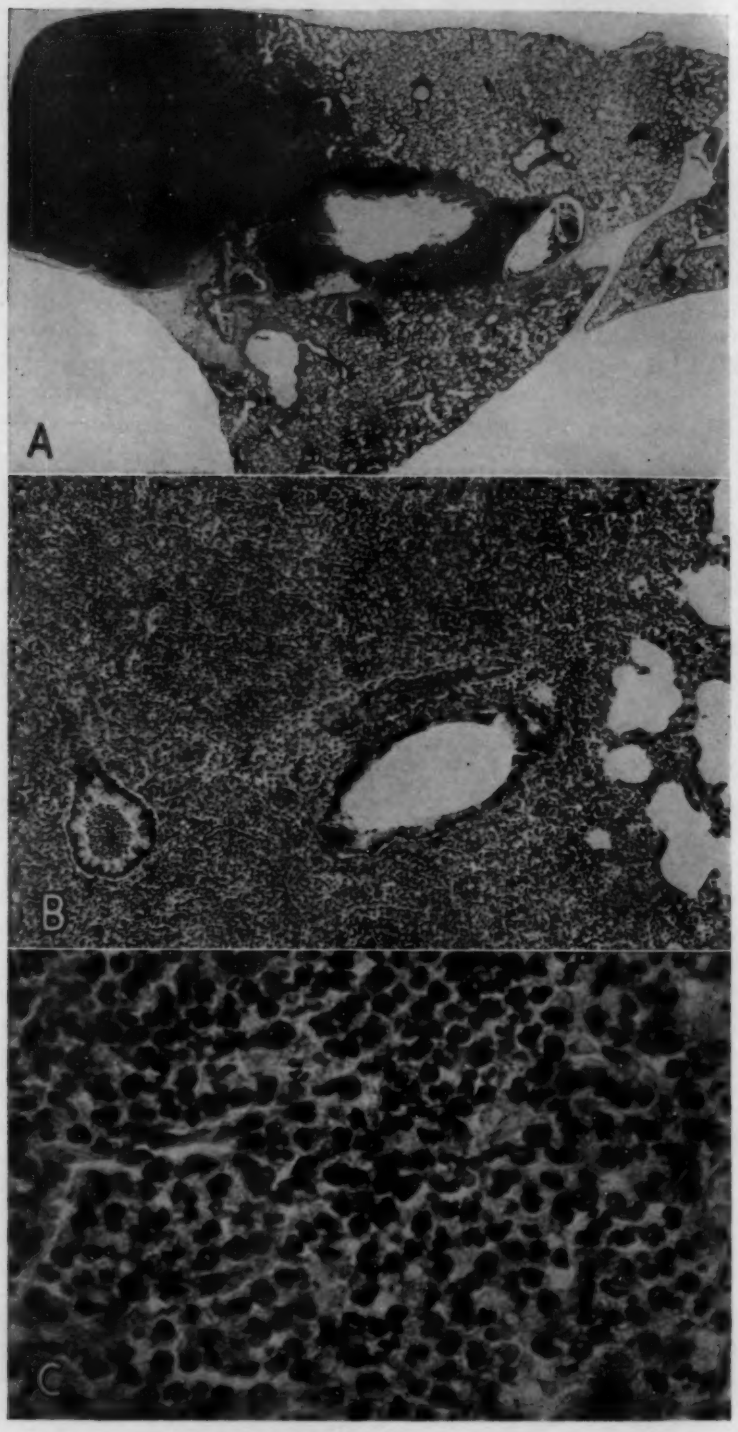

Figure 4

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eration of leukocytes. The chief finding was the absence of any appreciable evidence of resolution, as determined by dissolution of fibrin and appearance of mononuclear cells. For not only was fibrin abundant, but the polymorphonuclear leukocyte was the universal cell type, with 2 exceptions. In 2 rats there was found, in a single lobe of each, evidence of recovery in the form of localization of the exudate, scanty bacteria and moderate numbers of mononuclear forms. But both animals had fresh, spreading fibrinopurulent lesions in other lobes, and both had positive blood cultures. Abscess formation, pulmonary infarction and pulmonary thrombosis were each observed three times in this group of animals studied at four days.

The Wright-Giemsa demonstration of bacteria revealed myriads of diplococci in almost every field. Phagocytosis was rare; counts made on a small group of lung sections revealed that 1 to 2 per cent of leukocytes studied contained intracellular cocci.

4. *Evolution of Pneumonia in Rats Treated with Sulfanilamide.*—(*a*) At Twenty-Four Hours: Of 12 treated rats killed at twenty-four hours, there was grossly evident consolidation in 7, with an average involvement of one-half lobe. In microscopic appearance (fig. 4) these resembled the control lesions except for diminution in the extent of the lesion and sharper demarcation of the margins. The indefinite peripheral zone of red was usually absent.

In comparison with controls the treated animals revealed the following histologic differences: Leukocytes were present in the alveoli in more dense aggregates (fig. 4 *C*); they frequently showed earlier evidence of degeneration;[4] fibrin was present in smaller amounts and the nets were less coarse, and there was less of capillary engorgement

4. The earlier finding of degenerating forms may be due to a diminished influx of new or healthy leukocytes in an arrested inflammation.

EXPLANATION OF FIGURE 4

A, photomicrograph of a section of the lower lobe of the right lung of a treated animal that was killed at twenty-four hours, demonstrating the limited extent of the treated lesion and the abrupt transition between the pneumonia and the normal lung tissue; hematoxylin and eosin; × 12. (Compare this with the spreading lesion in figure 3 *A*.)

B, photomicrograph of a lung of a treated animal which was put to death at twenty-four hours. The exudate is fibrinopurulent, and there is purulent endobronchitis. The paucity of edema fluid and the absence of interstitial inflammation are to be contrasted with the pattern of the control rat in figure 3 *A*. Note the abrupt transition between pneumonic and normal lung; hematoxylin and eosin; × 75.

C, high power magnification of *B* to bring out in detail the compact exudate of polymorphonuclear leukocytes; hematoxylin and eosin; × 300.

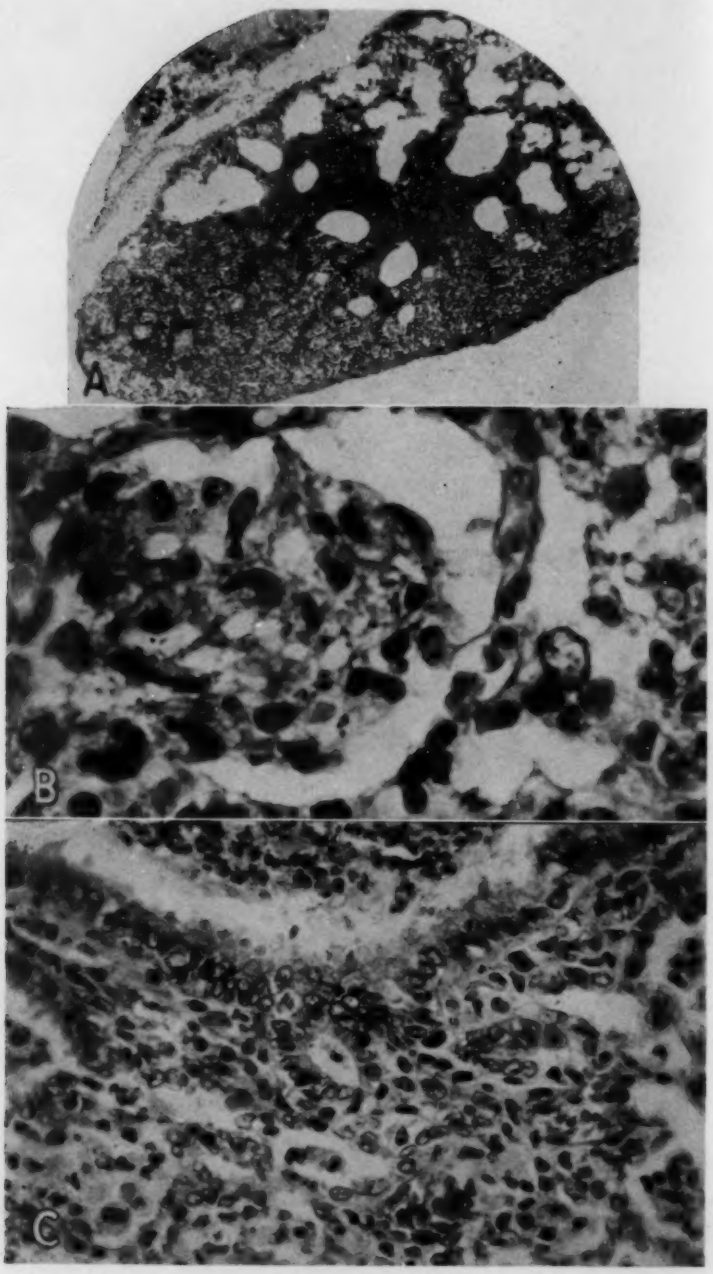

Figure 5

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and diapedesis of erythrocytes. Moreover, edema fluid was inconspicuous in amount and poor in protein in the treated group, whereas it saturated the parenchyma of involved areas in control material. The margins of the treated lesion bordered more or less abruptly on normal lung tissue; the peripheral zone of edema fluid seen in controls was absent, and interstitial inflammation of the septums was scanty or absent (fig. 4 *B*). This difference gave the treated lesion the appearance of being more sharply localized and thus provided a contrast with the spreading infection which so consistently characterized the controls.

All 12 treated animals exhibited lesions on microscopic study. These can be classified into three groups: those with diffuse fibrinopurulent exudate (6 rats); those with a patchy confluent lobular fibrinopurulent exudate (3 rats), and a doubtful group with patchy mononuclear exudate (3 rats). The difference between the first two groups was quantitative; they are classified apart because they presented noteworthy differences in the intensity of the inflammatory process invoked. The patchy lobular group (fig. 5 *A*) showed a less diffuse inflammation, in which fibrin was scanty and bacteria rare. The dominant cell type in both groups was the polymorphonuclear leukocyte. In the third group of rats a diagnosis of pneumonia could not be made. These animals accounted for three of the five grossly normal lungs encountered at twenty-four hours. The microscopic picture was characterized by focal intra-alveolar collections of mononuclear cells, large and small, without endobronchitis, bacteria or edema fluid. Lung cultures were sterile. The animals with the patchy mononuclear exudate differed from the mucin controls only in the extent of the reaction. An interpretation of their infections as abortive pneumonia could not be established with certainty.

When pulmonary sections of the 12 treated one day animals were studied under oil immersion, bacteria were found in relatively small numbers in 4 rats. Organisms appeared for the most part as extracellular cocci lying free in the alveoli. It is to be recalled that bacterial studies in control rats consistently revealed myriads of pneumococci. More-

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EXPLANATION OF FIGURE 5

A, view of a section of the lower lobe of the left lung of a treated animal that was killed at forty-eight hours, showing a limited and uneven confluent lobular pneumonia; hematoxylin and eosin; × 27.

B, photomicrograph of a field from *A*, to demonstrate the mononuclear predominance in the alveolar exudate; hematoxylin and eosin; × 200. There is moderate shrinkage of the exudate.

C, high power photomicrograph of a field from the lower lobe of the right lung of a treated rat which was killed at four days, showing purulent endobronchitis (with disintegration of leukocytes) and adjacent mononuclear alveolar exudate; hematoxylin and eosin; × 75.

over, forms undergoing phagocytosis were infrequent and were fewer than in controls. (Inability to demonstrate pneumococcic capsules in paraffin sections by our methods precludes comment on the influence of sulfanilamide on capsule formation.)

Four of the 12 treated rats received additional sulfanilamide in the standard dosage seventeen hours prior to operation.[5] The postmortem observations in these rats differed in no way from those in rats treated immediately after operation.

(*b*) At Two Days: Five of 8 rats treated with sulfanilamide and put to death at two days showed gross evidence of consolidation. The extent of consolidation averaged half a lobe, as at one day. Macroscopic pleural involvement was not seen at two days, in contrast to the observations in the two day controls, the majority of which exhibited empyema.

The microscopic observations in the lungs of the treated rats at two days gave the first evidence of a real change in the cellular composition of the exudate; most sections showed mononuclear cells widely distributed throughout the involved zones (fig. 5 *B*). Typically, there was observed diffuse confluent lobular pneumonia, the exudate of which contained fibrin, polymorphonuclear leukocytes and variable numbers of mononuclear cells. Some microscopic fields showed alveoli predominantly occupied by mononuclear cells; in these, bacteria were absent, and fibrin was scanty and appeared in granular form, suggesting that dissolution was in progress. Other fields revealed few monocytes, and here fibrin was more abundant. Bacteria were demonstrated in the fibrinopurulent areas of 3 of the 8 animals, always in very small numbers and rarely intracellular.

The group with patchy mononuclear exudate showed, with a possible single exception, true pneumonia, but on the whole the inflammatory process was less intense, with mononuclear cells overshadowing the polymorphonuclear cells in frequency. Purulent endobronchitis was common, however, to all two day rats, regardless of which cell predominated in the alveolar exudate, and this finding served not only to strengthen the common diagnosis of pneumonia but also to bridge the gap between the observations at one and two days.

Microscopic evidence of pleuritis was seen in only 2 of the two day animals. In both it was minimal in extent, and the mononuclear and polymorphonuclear leukocytes were present in these rats in approximately equal numbers.

In the treated group put to death at two days there were instances of well established macroscopic and microscopic fibrinopurulent pneu-

5. Interpolation from curves of blood levels of sulfanilamide in the normal rat, determined by Marshall and Cutting, indicates that the blood of our rats treated seventeen hours before infection contained approximately 10 mg. per hundred cubic centimeters of sulfanilamide at the time of infection.

monia involving an entire lobe in which bacteria could not be seen after twenty minutes of careful search.

(*c*) At Three Days: No treated animals were killed at three days.

(*d*) At Four Days: Of the 9 rats given sulfanilamide until put to death on the fourth day, 5 showed macroscopic involvement estimated to be the equivalent of one-half lobe. The consolidated zones typically were yellow-white (indicating a resolving process) and firm on section, with normal visceral pleura in all but 1 rat. Mononuclear cells were widely distributed throughout the exudate, and in many alveoli the fibrin had retracted from the walls. Additional evidence of resolution was found in frozen sections stained with sudan III, which revealed the presence of neutral fat within mononuclear cells. Purulent endobronchitis was still a common finding, but the polymorphonuclear leukocytes showed marked disintegration, while mononuclear cells were seen in small numbers. Bacteria were absent or rare, with few intracellular forms. In 2 rats of this group the pleural reaction was observed to be solely of mononuclear cells. Small abscesses were present in 3 animals.

The 4 rats showing focal mononuclear exudate presented somewhat the same problem in diagnosis as did their counterparts of two days. The common finding was a patchy confluent lobular aggregate of mononuclear cells with scanty or no fibrin. Bacteria could not be demonstrated in culture or section. The evidence in favor of the interpretation that these changes represented resolution of mild pneumonia was: the presence of bronchi devoid of mucous membrane and containing occasional leukocytes in their lumens; the finding of atelectatic alveoli with thickened septums, and, finally, the observation that these lesions were considerably more extensive than in the mucin controls.

(*e*) At Five, Six, Seven and Fifteen Days: This group of animals showed all stages of the recovery process: the substitution of a mononuclear for a fibrinopurulent exudate; the dissolution and disappearance of fibrin; the regeneration of bronchial mucous membrane; the development of pyogenic membranes about abscesses, and the fibroblastic organization of empyemas. In some instances, patchy atelectasis, thickened pleura and hyperchromatic cells lining bronchi were all the evidence that remained of preexisting pneumonia.

Consolidation was still grossly visible in 2 of the 8 rats put to death at fifteen days, involving most of the upper and lower lobes of the right lung in one, and half of the upper lobe of the right lung in the other.

Of the 6 rats in the fifteen day group whose lungs were grossly normal, 4 showed microscopic evidence of end stage resolution similar to that referred to in the foregoing paragraphs and in 1 rat there was mononuclear cell infiltration of the pleura. The other 2 rats offered no

clue to help decide whether or not these represented complete recovery from pneumococcic pneumonia.

Cultures of lung and blood were sterile, and study of preparations stained for bacteria failed to demonstrate pneumococci in any of the 8 animals.

5. *Mucin Controls.*—The 3 animals given sterile mucin alone were killed one on each of the three days after operation. All showed grossly normal lungs at autopsy, and cultures of lung and blood were sterile.

Microscopic study at one day revealed only a few intra-alveolar collections of mononuclear cells, mostly of the large septal cell type. There were a few polymorphonuclear leukocytes irregularly dispersed throughout, and fibrin was absent. The bronchi were normal save for the presence of mucin. By far the most important finding was the limited extent of the reaction.

At two days the reaction to mucin was reduced to barely perceptible proportions, and at three days the lung was regarded as normal.

6. *Pathologic Observations in Sulfanilamide-Treated Animals Which Died.*—Sulfanilamide failed to protect 4 animals against death from pneumonia, these deaths occurring on the third, fifth, eighth and eleventh days, respectively. Pulmonary cultures were positive for type 3 pneumococci in every instance, and the growth on blood agar was in appearance indistinguishable from controls.

The rats dying on the third and fifth days showed extensive lobar consolidation, which in gross and microscopic appearance closely resembled the changes observed in controls.

The rat dying on the fifth day showed serous effusion and such extensive lobar consolidation of the upper lobe of the right lung and the lower lobe of the left lung that the specimen was preserved for mounting. The blood culture was sterile.

The rat that died on the eleventh day received sulfanilamide for seven days after the introduction of pneumococci, and the animal remained well. After the administration of the drug was discontinued, there was a rapid downhill course to death, with respiratory embarrassment. The lung and blood cultures were positive for pneumococci. Postmortem study revealed empyema on the left side, overlying consolidation of the entire left lung. There was patchy involvement in the lower lobe of the right lung. On microscopic study there was fibrinopurulent pleuritis, rich in free diplococci. In the pulmonary parenchyma there was resolving pneumonia, with abundant mononuclear cells, little fibrin and no bacteria, and at the periphery of the old lesion was another displaying all the characteristics of fresh fibrinopurulent pneumonia. At the junction of the two processes was an old abscess in which bacteria could be demonstrated, and this probably served as the focus for the secondary spread, which unquestionably led to the death of the animal.

7. Pathologic Observations in Animals Given Sulfanilamide Thirty Hours After Infection.—In this experiment 7 rats were given treatment at thirty hours, the time at which the first control died. The remaining 2 controls died thirty-six and seventy-two hours after infection.

Four of the treated rats died, 2 of them shortly after the first treatment, and 1 on the third and 1 on the seventh day. The 3 survivors appeared clinically well on the eighth day, at which time they were put to death.

The observations on the 3 treated rats whose deaths occurred in the first three days were similar to those on the controls in all respects; fibrinopurulent pneu-

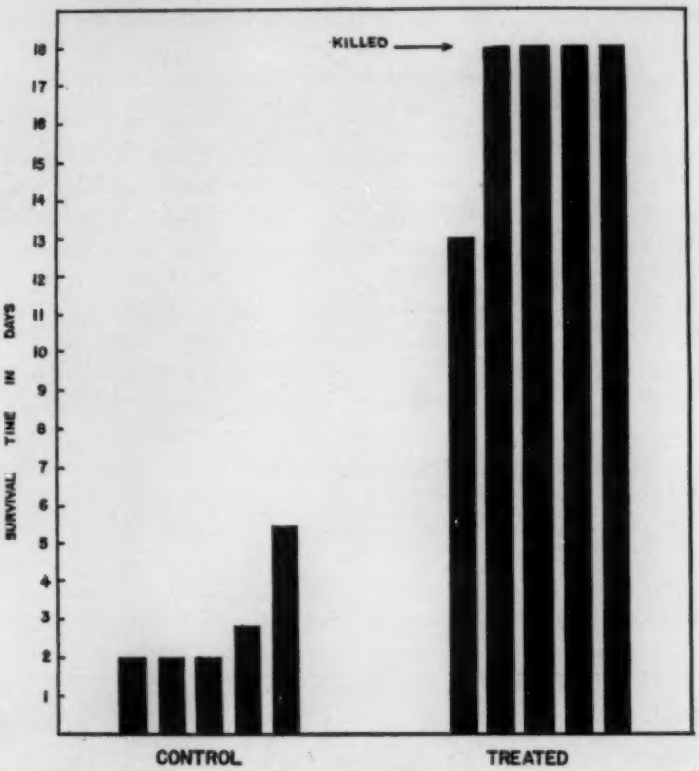

Fig. 6.—Effect of sulfapyridine on survival. Each column represents 1 rat. Each rat received 0.3 cc. of a 1 : 10 dilution of a culture of type III pneumococci in mucin by the transtracheal route. Each treated rat received 200 mg. of sulfapyridine daily through the seventeenth day.

monia, pleural involvement and the recovery of pneumococci from lung and blood were findings common to all. The rat dying on the seventh day exhibited extensive consolidation of the upper lobe of the right lung, empyema and peritonitis. Cultures were positive. Microscopic study showed resolution in progress; the monocyte was the predominant cell in the empyema membrane; a small number of bacteria were seen, a few of which were intracellular.

The 3 animals which appeared well when killed on the eighth day showed gross consolidation ranging from one half of a lobe to an entire lung. All showed

fibrinopurulent pneumonia undergoing resolution and a fibroblastic response in the pleura. Bacteria could not been seen in sections; cultures of lung and blood were sterile.

OBSERVATIONS IN EXPERIMENT WITH SULFAPYRIDINE

1. *Effect on Survival Time (Fig. 6).*—All 5 controls died prior to the sixth day. As seen in figure 6, 4 of the 5 treated rats survived until put to death on the eighteenth day. Treatment was begun shortly after infection and continued once daily until the fifteenth day. The single death among the treated rats (fifteenth day) was caused by mediastinitis

TABLE 2.—*A Composite of the Pathologic and Bacteriologic Observations on Sulfapyridine-Treated and Control Rats Put to Death at One, Two and Eighteen Days*

Experiment	Rats	Extent of Pneumonia	Microscopic Change	Pleural Involvement	Positive Cultures		Microscopic Estimate of Bacteria
					Lung	Blood	
Controls killed after 1 day	4	2 ++ 2 ++++	Diffuse fibrinopurulent exudate	3+	4	1	++++
Treated rats killed after 1 day	8	4 + 3 ++ 1 none	5 with diffuse fibrinopurulent exudate 1 with fibrinopurulent and mononuclear exudate 1 with patchy mononuclear exudate 1 none	0	6	0	4 + 1 ++ 3 none
Controls that died on 2d day	4	4 ++++	Diffuse fibrinopurulent exudate	4+	4	4	++++
Treated rats killed on 2d day	8	5 ++ 1 +++ 2 none	6 with diffuse fibrinopurulent and mononuclear exudate 2 none	1+	1	0	8 none
Treated rats killed on 18th day	4	4 none	4 with patchy mononuclear exudate (1 organizing)	0	0	0	4 none

resulting from an accident in tube feeding. The results of the pathologic and bacteriologic studies are seen in table 2.

2. *Comparison of Pathologic Changes in Controls with Those in Treated Animals at One and Two Days.*—Table 2 is a composite of the observations made in the sulfapyridine study; it represents the pooled findings in two experiments, which included 10 controls and 21 treated rats. The results, both morphologic and bacteriologic, differed in no way from those in the experiments with sulfanilamide. The number of animals employed in the survival study is not large enough to permit a precise comparison of the efficacy of the two compounds. Certain it is that both compounds appear highly effective under the conditions of our experiments and influence in an identical manner the evolution of experimental type 3 pneumococcic pneumonia in the rat.

OBSERVATIONS ON VISCERA OTHER THAN THE LUNGS OF CONTROL AND TREATED RATS

Careful study of preparations of bone marrow from control and sulfanilamide-treated rats failed to demonstrate any significant deviation from normal.

Sections of the spleen, liver and kidney of each animal were examined and found remarkably free of pathologic change. In 1 instance of protracted administration of sulfapyridine inspection of the renal pelves disclosed multiple pinhead-sized calculi, but microscopic study showed normal renal structure.

Pericarditis was frequently observed in rats dying with empyema. A routine study of the cardiac valves was not made.

COMMENT

The administration of sulfanilamide and sulfapyridine altered the evolution of the experimental inflammation and reduced the mortality; the treated pneumonia was much less extensive, there was no spread of the lesion after twenty-four hours, and there was marked reduction in the incidence and severity of pleural involvement. Pleural involvement occurred in over 90 per cent of the controls, in contrast to 20 per cent of the treated rats. Frank empyema was noted in 75 per cent of the controls and in less than 5 per cent of the treated rats. However, abscess formation occurred almost as frequently in the treated as in the control group (25 per cent), while the incidence of vascular thrombosis and pulmonary infarction was 25 per cent in the controls and 10 per cent in the treated animals. Bacteremia was noted once in the treated group, whereas the blood cultures of the controls were regularly positive for pneumococci at death and frequently positive at twenty-four hours after infection.

At twenty-four hours the chief cells of the exudate were polymorphonuclear leukocytes, while after forty-eight hours, in most instances of treated pneumonia the mononuclear cells appeared to dominate the alveolar exudate. (This increase in numbers of mononuclear cells at this stage may be a relative rather than an absolute increase, in the light of the decreased numbers of polymorphonuclear leukocytes.) With the appearance of the mononuclear form there occurred solution of fibrin, thinning of the intra-alveolar exudate and resolution of the pneumonia. But the most striking observation in rats treated with sulfanilamide or sulfapyridine was the early disappearance of bacteria both in cultures and in stained sections. This was noted as early as twenty-four hours and in instances of well established fibrinopurulent pneumonia. Moreover, fibrin and edema fluid were most abundant in those lesions in which bacteria were most numerous, while in those sections in which the mononuclear cells were prevalent bacteria were absent and fibrin was scanty, often in granular form. It was not possible to make observations on capsular changes from histologic preparations. However, pneumo-

cocci were recovered from the lungs of 19 treated rats, and these grew in blood agar as smooth, mucoid colonies and gave a positive Neufeld reaction.

Since treated rats at twenty-four hours showed a dense fibrinopurulent exudate similiar to the exudate of the controls the changes in the treated inflammatory pattern noted after this period must represent an evolutionary process starting from the same stage as in untreated animals.

Because of the disappearance of bacteria under the influence of sulfanilamide and sulfapyridine, the role of phagocytosis requires analysis. Phagocytosis of pneumococci was infrequently seen in control lungs and was observed with greater rarity in the sections of treated lungs. It seems safe to state that phagocytosis appeared to play no significant part in the disappearance of bacteria observed in the treated rats. This is in agreement with the observations made by Long, Bliss and Feinstone[6] in type III pneumococcic peritonitis in the mouse treated with sulfanilamide. They stated: "It is noteworthy that the observed bacteriostasis is unaccompanied by any especial degree of phagocytosis."

As interesting as the absence of phagocytosis in the treated animals was the insignificant phagocytosis in the controls. Cooper and Gross[7] made similar observations in their work on experimental type III pneumococcic pneumonia.

The overwhelming dose of pathogenic micro-organisms used may have altered the capacity of the polymorphonuclear leukocytes for phagocytosis. Yet even in those killed treated animals which showed sharp reduction of bacterial numbers there was no appreciable phagocytosis. Although each lesion in this study was searched for intracellular organisms, both in spreads from fresh cut surfaces of the affected lung and in paraffin sections, phagocytosis of bacteria was seldom found. In the light of this finding it seems profitable to reinvestigate the entire subject of the role of phagocytosis in inflammation due to the pneumococcus. While Robertson[8] assigned a protective role to the microphage and the macrophage by virtue of their capacity for phagocytosis, Gunn and Nungester[1] suggested that leukocytes bearing intracellular cocci may, by active migration, bring about spread of the pneumonia.

The role of the mononuclear cells found in experimental pneumonia has attracted considerable attention. Robertson and his co-workers,[8] in their studies of the pneumonic lesions of untreated dogs, observed at

6. Long, P. H.; Bliss, E. A., and Feinstone, W. H.: J. A. M. A. **112**: 115, 1939.

7. Cooper, F. B., and Gross, P.: Proc. Soc. Exper. Biol. & Med. **36**:678, 1937.

8. (*a*) Robertson, O. H.: J. A. M. A. **111**:1432, 1938. (*b*) Robertson, O. H., and Coggeshall, L. T.: J. Exper. Med. **67**:597, 1938. (*c*) Robertson, O. H., and Loosli, C. G.: ibid. **67**:515, 1938.

the time of recovery a cellular change characterized by a mononuclear cell response, which they have designated the "macrophage reaction." They, like others before them, noted that the macrophage was found as a constant accompaniment of recovery. They consider the mononuclear cells capable of destroying pneumococci much more effectively than the polymorphonuclear leukocytes. These authors made the observation, confirmed by this study, that "pneumococci were found in relatively small numbers in the lesions" at the recovery stage. "They were more frequent where the polymorphonuclears predominated, and less numerous or absent in the areas of macrophage reaction." The inference is made that bacteria were rare in macrophage zones because of the phagocytic activity of the mononuclear cells.

Gay and Clark [9] reported "a precocious and increasing mobilization of clasmatocytes" in the parietal and visceral pleura of rabbits given streptococcic empyema and treated with sulfanilamide. They stated further that "there is direct evidence that the drug does not in itself stimulate the mobilization of macrophages." But they further wrote, "In other words, sulfanilamide apparently produces a bacteriostasis sufficiently marked to protect the accumulated leucocytes and to allow the natural defense macrophages to accumulate." They left the inference that the disposal of the organisms is thereby effected or enhanced.

Two findings in our experimental pneumonic lesions suggest that the appearance of the mononuclear cells was not the cause of the disappearance of the bacteria. First, in the treated group there was marked diminution in the numbers of bacteria to be found twenty-four hours prior to the appearance of mononuclear cells, when the cellular exudate was universally polymorphonuclear. Second, phagocytosis of bacteria by mononuclear cells was not observed in this work. There appears definitely to be a time relation between the disappearance of bacteria and the presence of mononuclear cells, but, further than this, to assign a defensive or protective role to the macrophage on the evidence at hand seems unwarranted teleology.

The relation of the action of the drugs used to concomitant or secondary formation of antibodies was not studied. However, there is ample evidence in Whitby's [10] work on pneumococcic infections in mice and Finland's [11] studies in man to support the tenet that sulfanilamide and sulfapyridine do not in themselves promote or accelerate the production of antibodies. Since phagocytosis did not appear to play a significant role in the disappearance of the bacteria, and since there was appreciable diminution in the numbers of pneumococci in treated animals at a time

9. Gay, F. P., and Clark, A. R.: J. Exper. Med. **66**:535, 1937.

10. Whitby, L. E. H.: Lancet **2**: 1095, 1938.

11. Finland, M., and Brown, J. W.: J. Clin. Investigation **18**:307, 1939.

when the cellular character of the exudate was like that seen in untreated animals, the mode of action of sulfanilamide and sulfapyridine appears to be directly on the bacteria, effecting bacteriostasis and possibly bacteriolysis, in the broadest meaning of these terms.

The effect of the drugs used may be summarized by saying that the experimental inflammation was limited and rendered free of bacteria. The recovery process, not seen in controls (owing probably to overwhelming dosage), was initiated. Since resolution appeared earlier in our treated rats than it did in the untreated animals reported by Gunn and Nungester,[1] receiving very dilute infecting doses of type III pneumococci, acceleration of the recovery process should be added to the effects of the administration of sulfanilamide and sulfapyridine. The histologic pattern seen in our treated rats was characterized by early and preponderant appearance of mononuclear cells after twenty-four hours. These cells have been shown to be a constant accompaniment of recovery in pneumococcic pneumonia in man and in animals, even in the absence of treatment.[12]

SUMMARY

Sulfanilamide or sulfapyridine administered in rats shortly after intratracheal injection of lethal doses of type III pneumococci lowered mortality and promoted recovery with approximately equal effectiveness. In the treated animals as compared with the controls, the extent of the pneumonia was limited and the evolution of the inflammatory pattern was altered, principally by the early appearance of mononuclear cells. In the treated rats there was in most instances early disappearance of pneumococci from the lung, and no bacteremia.

The incidence of frank empyema was 75 per cent in the controls and 5 per cent in the treated animals. The untreated rats exhibited a 25 per cent incidence of abscess formation, pulmonary thrombosis and pulmonary infarction. The treated rats showed the same incidence of abscess formation and a 10 per cent incidence of thrombosis and infarction.

The disposal of bacteria in the treated animals did not appear to be related to phagocytosis.

12. Since the completion of this report Gregg, Hamburger and Loosli have published the results of their investigation of sulfapyridine in experimental pneumococcic pneumonia in the dog (J. Clin. Investigation **19**:257, 1940). The findings in our studies in the rat are in close agreement with the findings reported by these authors.

SKIN COLOR AND SKIN CANCER

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A commonplace in dermatologic lore is that skin cancer occurs more frequently in blonds than in brunets. Supporting this impression are (1) the extremely low incidence of the condition in the deeply pigmented races, such as Negroes and American Indians; (2) the high coincidence with xeroderma pigmentosum, a disease of patients whose skin is very fair and unusually sensitive to sunlight, and (3) the fact that skin cancers most frequently occur on surfaces of the body habitually exposed to the actinic rays of sunlight.

Among others, Molesworth [1] found that, of 37 Northern Europeans with skin cancer, only 2 were pronouncedly brunet. Voltz [2] wrote, *Der rotblonde Menschentypus ist offenbar für Karzinom leichter disponiert, als andere Menschentypen. . . . In den letzten fünf Jahren uns zugegangenen jugendlichen Karzinom-trägerinnen (unter 35 Jahren) etwa 70 Proz. dem rotblonden Menschentypus angehörten* (The reddish-blond type of person apparently is more predisposed to carcinoma than are other human types. . . . Of the young persons with carcinoma [under 35 years] who came to us during the last five years, approximately 70 per cent were of the reddish-blond type). Both of these observers made their judgments of skin color without instrumental aid. The subjective visual estimation of skin color obviously will vary with each observer and even with different observations by the same investigator.

Epstein [3] tried to solve the difficulty by classifying his subjects on the basis of hair color and eye color, assuming a correlation between these expressions of pigmentation and that of skin color. For the purpose in mind, the assumption of a perfect or even very high correlation between any two of these is unwarranted. Thus Yule [4] gives the

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This investigation has been aided by a grant from the Division of Medical Sciences of the National Research Council, Washington, D. C.

1. Molesworth, E. H.: Urol. & Cutan. Rev. **31**:543, 1927.
2. Voltz, F.: München. med. Wchnschr. **78**:48, 1931.
3. Epstein, S.: Arch. f. Dermat. u. Syph. **164**:304, 1931.
4. Yule, G. U.: An Introduction to the Theory of Statistics, ed. 8, revised, London, Charles Griffin & Co., Ltd., 1927, p. 66.

mean square contingency coefficient [5] for association of hair and eye color in a series of 6,800 Germans of Baden (data quoted from Ammon) as 0.37. One of the writers found a coefficient of 0.51 for association of eye color with skin color in a series of 133 American Negroes. There is no doubt that hair, skin and eye color are related in expression, but not to a predictable degree.

Various instrumental aids to the determination of skin color have been devised. The first good semiobjective aid was that of Broca,[6] reprinted on a larger scale by Hrdlicka,[7] consisting of a series of tints printed on paper. One great objection to this scheme was that the colors faded on exposure to sunlight. The next step forward was that of von Luschan, who made his tint series in porcelain. These colors appear to be permanent but unfortunately are of little help in determining cutaneous pigmentation of whites, although good matches can be made with more deeply pigmented skins. A further objection to the von Luschan scale is that the subjective element is not eliminated. However, its easy portability and relative objectivity (as compared with guessing and verbal description) have made it an instrument much used by anthropologists. Todd and van Gorder [8] recommended use of the color top. However, the colors (on paper) are subject to fading, the method takes considerable time, and the results are strongly subjective.

The visual spectrophotometer was used in the determination of skin color by Brunsting and Sheard.[9] This instrument is superior to all devices so far mentioned in that the determination is purely objective, is stated in terms of wavelength and reflectance and is based on an unvarying light source. Hardy's [10] perfection of the recording photoelectric spectrophotometer marks the ultimate development in a nonportable laboratory instrument for the analysis of color. It has been used for the determination of skin color by Edwards and Duntley.[11] The recording spectrophotometer is far superior to any other instrument for descriptive expression of skin color. However, it has two disadvantages: it is expensive and nonportable.

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5. The coefficient of correlation is a statistical device whereby relationship is expressed on a quantitative scale. The coefficient of mean square contingency is a method by which two qualitative estimates or a qualitative and a quantitative estimate may be related.

6. Broca, P. P.: Instructions générales pour les recherches anthropologiques à faire sur le vivant, ed. 2, Paris, G. Masson, 1879, vol. 2.

7. Hrdlicka, A.: Directions for Collecting Information and Specimens for Physical Anthropology, Bulletin 39, part R, United States National Museum, 1904.

8. Todd, T. W., and van Gorder, L.: Am. J. Phys. Anthropol. **4**:239, 1921.

9. Brunsting, L. A., and Sheard, C.: Proc. Staff Meet., Mayo Clin. **4**:110, 1929.

10. Hardy, A. C.: J. Optic. Soc. America **25**:305, 1935.

11. Edwards, E. A., and Duntley, S. Q.: Am. J. Phys. Anthropol. **23**:503, 1938.

INSTRUMENTAL TECHNIC

One of us consulted Hardy, seeking a device satisfactory for the determination of skin color which would be portable and not so expensive as the spectrophotometer. A characteristic of skin colors is that they are not highly saturated. In view of this, Hardy advised that a portable trichromatic colorimeter would fill the need. Such a colorimeter was built under his direction and was described by Williams.[12]

The essential characteristics of the instrument are as follows:

Its objective end is a spherical cavity illuminated by four automobile headlight bulbs. At one end of the sphere is an opening at which the skin or object to be examined is placed and next to it within the sphere is a plaque of magnesium carbonate. Light is reflected both from the object and from the plaque of magnesium carbonate to the opposite end, where each set of rays enters a series of lenses and prisms, finally passing through Wratten filters of red or green or blue color. On looking into the ocular, one sees that the field is divided into two parts, one half showing light reflected from the object, the other half showing light reflected from the magnesium carbonate plaque. By turning the vernier, the light from the magnesium carbonate is diminished by use of a Nicol prism so that the two halves of the field exactly match, as in any colorimeter. Readings are taken through each of the filters. (Owing to the fact that skin colors are not highly saturated, the color difference in the two halves of the field is ordinarily small, and readings are but little affected by anomalies in the observer's visual mechanism.)

Preliminary to taking a reading on skin, the instrument is calibrated for each filter by placing the window of the sphere over a freshly scraped block of magnesium carbonate. The reflecting power of the skin relative to that of the standard white magnesium carbonate block is then determined for each filter by calculating the ratio of the tangent squared of the angle read on the skin to the tangent squared of the angle read on the magnesium block. The ratio is expressed in terms of percentage.

MATERIAL AND METHOD

The cancerous subjects of this study were all patients of the Barnard Free Skin and Cancer Hospital. In each case the diagnosis was confirmed by biopsy. The series consisted of 100 consecutive patients, 67 males and 33 females. For controls, age mates of the cancerous patients were examined at the City Infirmary, St. Louis, and at the St. Louis City Hospital. Seventy-one of these were males and 29 females. They were all noncancerous and were hospitalized for other conditions. None of them was very ill at the time of examination.

The skin of each subject was examined with the colorimeter described in three regions—the forehead, the medial side of the arm and the back just superior to the buttocks. The choice of these regions was based on the belief that the forehead is most exposed to the effects of sun and weather, the arm less and the back least. The back, therefore, was presumed to exhibit the closest approximation to congenital skin color.

12. Williams, G. D.: Science **78**:192, 1933.

RESULTS

When the aperture of the colorimeter is placed on the standard magnesium carbonate and no filter is interposed, white light, i. e., light of all visible wavelengths, is reflected from it to the eye piece. If the red filter is interposed, only the red light from the magnesia is permitted to pass it. This amount of red light passing through the red filter is taken as the standard, or the maximum amount of red that can be reflected from a surface. An area of skin also reflects light waves but of varying wavelengths in varying amounts. If a red filter is used, only the red of the skin is reflected to the eye piece. Therefore, in making a reading with the colorimeter on skin, the light from the comparison plaque is decreased by turning the Nicol prism of the photometer until the eye agrees that its intensity equals that reflected from the skin. The more the prism must be turned away from the magnesium standard reading to obtain a match of the colors, the less is the amount of red

TABLE 1.—*Skin Color of Forehead (Colorimeter)*

	Number in Group	Red	Green	Blue
Males				
Controls	71	46.51 ± 0.49	28.40 ± 0.42	26.12 ± 0.35
Cancerous patients	67	44.22 ± 0.45	25.92 ± 0.36	24.66 ± 0.41
Difference		2.29 ± 0.66	2.48 ± 0.55	1.46 ± 0.54
Females				
Controls	29	49.09 ± 0.69	30.33 ± 0.66	28.33 ± 0.57
Cancerous patients	33	48.95 ± 0.73	29.32 ± 0.76	27.68 ± 0.67
Difference		0.14 ± 1.00	1.01 ± 1.01	0.65 ± 0.88

that is reflected from the skin. The exact proportion is obtained as a percentage by calculation. The same is true for the two other filters, the green and the blue. If more light is passed through *all* filters by one skin than another, it means that the first skin is lighter than the second or that the second is darker than the first.

Thus, in comparison of the sexes it was found that higher average percentage readings were obtained through all three filters for both cancerous patients and controls on the foreheads of females than on those of males (table 1). This is not true of the readings on their arms and backs. It simply means that the foreheads of males are darker than those of females, probably because of greater exposure to sun and weather, and that the arms and backs, being relatively unexposed in both sexes, do not so differ. It will be necessary, then, in comparison of cancerous patients and controls, to segregate the data for the sexes in the case of the forehead readings. However, in the case of arm and back determinations, the data for the two sexes can be pooled.

Means and standard errors of the forehead readings for the cancerous and control groups, divided according to sex, are given in table 1.

When a difference exceeds three times its standard error, that difference is said to be statistically significant. In regard to males, significant differences between cancerous persons and controls are discovered in the readings of skin color transmitted through the red and green filters. In regard to females, the differences are all insignificant. This means that the foreheads of cancerous males are definitely darker than those of control males. The females, both cancerous and controls, have lighter foreheads than the corresponding males. Their foreheads have not been exposed to sun and weather as much as have those of the men, just as the foreheads of the male controls have not been exposed as much as have those of the cancerous males (table 2). One should therefore expect the sex difference to be greater in cancerous persons than in controls. This is the case as shown in the differences of table 2.

Referring again to table 1, it was calculated that the cancerous-control difference in males is three times its standard error through the red filter, twice the error through the blue and four times through the green. Thus, although significantly more red light is reflected from the fore-

TABLE 2.—*Sexual Differences in Skin Color (Based on Table 1)*

	Red	Green	Blue
Controls	2.58 ± 0.85	1.93 ± 0.78	2.21 ± 0.67
Cancerous patients	4.73 ± 0.86	3.40 ± 0.84	3.02 ± 0.79

heads of the controls than from those of the cancerous patients, even more green is so reflected. So that proportionately there is more green and less red in the forehead skin of the controls than there is in that of the cancerous persons. It has already been demonstrated that the cancerous persons' foreheads are darker; i. e., they reflect less light of all wavelengths. It is now apparent that there is less green in proportion to red in the skin of this group. Therefore, since red is the complement of green, the cancerous persons' foreheads appear to the eye not only darker but also redder.

Readings on the medial surface of the arm show no sexual difference. With the red and the blue filters, no differences were found between cancerous patients and controls. However, determinations by means of the green filter show a difference between cancerous and noncancerous males of 2.65 ± 0.78, a difference of more than three times its standard error and statistically significant. Thus the arms of normal persons reflect more green light than those of cancerous patients. Therefore the latter, reflecting less green, appear redder. The female group shows no difference between the cancerous patients and the controls.

Readings on the back show neither differences between the sexes nor differences between cancerous persons and controls.

A difference in skin color of forehead and arm has been demonstrated between the cancerous and the noncancerous. There are several varities of skin cancer. Are they all similarly characterized by darker and redder foreheads and arms? To investigate this point, the cancerous group was further subdivided according to type of lesion into: (1) the basal type, including basal cell, basal cell adenoides and cystic basal cell; (2) the squamous type, including squamous cell and malignant horn; (3) the mixed type, including mixed cell and hair follicle cancers,

TABLE 3.—*Division of Male and Female Cancerous Groups into Cancer Type Subgroups*

Type	Males			Females		
Squamous	27			5		
Basal	27	34	40	15	21	28
Mixed	7			6		
Basosquamous	6			7		
Total	67			33		

TABLE 4.—*Differences in Skin Color of Forehead (Colorimeter) Between Males of Control Group and Males of Cancer Type Subgroups*

	Number in Group	Red	Green	Blue
Males				
Controls	71	46.51 ± 0.49	28.40 ± 0.42	26.12 ± 0.35
Basal	27	43.80 ± 0.63	25.57 ± 0.40	24.02 ± 0.63
Basal + mixed	34	43.74 ± 0.65	25.15 ± 0.47	24.09 ± 0.56
Nonsquamous	40	43.90 ± 0.58	25.10 ± 0.41	24.25 ± 0.52
Squamous	27	44.69 ± 0.69	27.13 ± 0.58	25.28 ± 0.62

and (4) the basosquamous cell type. The distribution of the types in the 100 patients is shown in table 3.

Comparison of skin color readings made on the foreheads of males yields the differences shown in table 4.

Calculation shows the following differences to be significant: the control — basal, the control – basal + mixed, the control — nonsquamous. This is true for all three filters. The differences between the control group and the squamous group are all insignificant, although that obtained using the red filter is large enough to be twice its standard error. Furthermore, it is discovered that the differences are greatest between controls, on the one hand, and the basal and mixed cancer type groups, on the other, in readings through the green filter. These differences (in readings made through the green filter) exceed their respective standard errors by four or five times, while those taken through the other filters exceed their errors by no more than three times. The conclusions

drawn concerning differences in forehead skin color between cancerous and noncancerous subjects may now be further refined: The foreheads of male patients having the basal and mixed types of cancer are darker and redder than those of noncancerous subjects, but no such difference characterizes patients with the squamous type of cancer. This difference in skin pigmentation between squamous and other skin cancer types applies only to males, no significant differences being found in females.

It has been determined that skin color on the medial surface of the arm shows no sexual difference in either cancerous or control groups. In table 5 are presented the pooled data for the sexes on skin color in the noncancerous group and in the various types of cancerous subjects.

With the red filter no significant differences are discovered; larger differences are found between the mean readings with the blue filter, but none of them is significant. But with the green filter the same list of significant differences appears as in the case of readings on the

TABLE 5.—*Differences in Skin Color of Arm (Colorimeter) Between Control Group and Cancer Type Subgroups*

	Number in Group	Red	Green	Blue
Both Sexes				
Control	100	60.34 ± 0.46	44.26 ± 0.47	41.60 ± 0.52
Basal	42	59.36 ± 0.78	41.21 ± 0.67	39.64 ± 0.75
Basal + mixed	55	59.65 ± 0.64	41.32 ± 0.58	39.83 ± 0.68
Nonsquamous	68	58.97 ± 0.60	41.09 ± 0.53	39.44 ± 0.62
Squamous	32	60.56 ± 0.66	43.56 ± 0.81	41.44 ± 0.80

forehead: the control – basal, the control – basal + mixed and the control – nonsquamous. The control – squamous difference is insignificant. Differences between the squamous and the other cancer types are insignificant through red and blue filters, but the corresponding means through the green filter do differ to the extent of twice the respective standard errors. One may conclude that the arms of all patients with skin cancer except those having the squamous type are redder than those of the controls.

The readings taken on the back reveal no significant differences between any of the groups. Whether cancer is absent or present in any type, on the average the color of the skin on the back does not vary.

It is noteworthy that neither on the forehead nor on the arm nor on the back does skin color vary in females. Only males exhibit the group differences discussed. We have already suggested that the reason for the appearance of significant differences in males and not in females is the relative lack of exposure of the most exposed parts (the forehead and arm) in women. In and about St. Louis women are indoor workers to a much greater extent than are men. It is logical to inquire if there

is a difference in this respect between cancerous and noncancerous men. To determine this, the cancerous and noncancerous groups of males were subdivided into indoor and outdoor workers on the basis of their stated occupations. Thus the outdoor workers included farmers, laborers, carpenters, peddlers, gardeners and teamsters, while the indoor group comprised such occupations as coal miners, waiters, mechanics, musicians, clerks, iron workers, barbers, janitors and railroad conductors.

Division of the male cancerous and control groups into the two categories showed that of 66 cancerous men, 39, or 59.1 per cent, were outdoor workers, while of the 71 noncancerous men, only 23, or 32.4 per cent, had outdoor occupations. The percentage difference is 26.7, with a standard error of the difference of 8.51. The difference exceeds its standard error by more than three times and is therefore significant. More subjects with skin cancer than without have been unusually exposed to sun and weather.

TABLE 6.—*Division of Male Cancer Type Groups into Outdoor and Indoor Occupational Groups*

Cancer Type Group	Outdoor Workers	Indoor Workers
Squamous	17	10
Basal	16	10
Mixed	3	3
Basosquamous	3	4
Total	39	27

The 66 cancerous males may be grouped as to cancer type and nature of occupation as shown in table 6.

The numbers in each category are too small for trustworthy statistical manipulation, but there appears to be no great difference between the squamous and the other skin cancer groups in outdoor exposure. It appears likely, therefore, that patients with squamous and patients with nonsquamous types of skin cancer were equally exposed to the outdoor environment, but that the exposed skin of patients with the squamous type reacted differently from that of patients with other varieties of skin cancer. Or, one may conclude that persons with the basal type react to outdoor exposure by reddening and darkening of the exposed skin, a phenomenon which does not occur in persons having the squamous type; and that the exposed skin of the latter type resembles in lack of reaction that of noncancerous persons who may or may not have been greatly exposed to outdoor environment.

The subjects of this study—both cancerous and noncancerous—are almost exclusively of Middle or North European origin. They are therefore representative of a population which exhibits less cutaneous

pigmentation than any other human group. Skin cancer is more common in lighter than in darker-skinned peoples. The reason may be that the latter have a more effective protective mechanism in the ability to form skin pigment. The discussion of this matter lies outside the province of this paper for two reasons: (1) control data on darker white or on colored peoples are lacking; (2) the potentialities of the various subjects for increasing skin pigmentation through controlled exposure to sunlight or artificial light are unknown.

Discussion of the available data does, however, bring to light a fact of great interest—that the skin color of the back is on the average the same for all groups, whether the cancerous or the noncancerous, the squamous or the nonsquamous in cancer type. This finding is at variance with the hypothesis that the victims of skin cancer are congenitally different in skin color from the remainder of the white population. In the ordinary mode of life of the 200 subjects here studied, the lower part of the back is seldom exposed to the outdoor environment. The differences in skin color that have been demonstrated are those found on either the forehead or the arm. The skin color of the unexposed back is as blond in one group as in another. Therefore, no relationship exists between the color of unexposed skin of this group of white people and the incidence of skin cancer.

It has been shown that persons with skin cancer were more exposed to sun and weather in their occupations than were an unselected group of hospitalized age mates. It may be suggested that such exposure has some causal connection with the development of skin cancer. In the cancerous group it was found that on undue exposure some skins redden, or redden and darken; others do not. Those who showed the reddening reaction (sailor's skin of Unna) tend to have the basal type of cancer, while those who do not so react are more likely to show the squamous variety. In other words, some skins react differently to the stimulus of exposure than do others, even though they are congenitally similar. It is interesting that this difference in reaction appears to be correlated with a difference in the type of cancer that develops.

SUMMARY

The skin color of the forehead, the arm and the back was determined by use of a colorimeter in 100 unselected patients with skin cancer and 100 noncancerous hospitalized age mates. Statistical examination of these determinations showed:

The exposed skin of the foreheads and of the arms of patients with skin cancer was on the average definitely redder and darker than that of the noncancerous. The unexposed skin of the backs showed no such difference.

Information concerning the occupations of the male subjects indicated that patients with skin cancer were more often exposed to the action of the external environment than were the controls. This suggests a probable causal connection between exposure of skin and development of skin cancer.

Subjects whose skins on unusual exposure tend to darken and redden, if they show skin cancer, more often exhibit the basal or the mixed types. Those who do not so react to unusual exposure more frequently show the squamous variety if cancer develops.

STUDIES IN EXPERIMENTAL LIPOIDOSES

I. PHOSPHATIDES

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The term "primary or essential lipoidosis" is used to denote a group of diseases characterized pathologically by an infiltration of various tissues and organs with abnormally high amounts of lipoids. Following a widely accepted classification based on the chemical properties of the lipoid cell content, three main types can be considered: (1) lipoidosis due to phosphatides, in which the infiltrating lipoid consists of sphingomyelin; (2) lipoidosis due to cerebrosides, in which kerasin makes up the substance loading the cells, and (3) lipoidosis due to cholesterol, in which esterified or free cholesterol is found in the altered tissues.

Although numerous studies have greatly enlarged the knowledge of these diseases, many problems remain open for further investigation. It is the purpose of the present work to contribute to the study of lipoidosis from an experimental point of view. To be sure, much literature on experimental cholesterol lipoidosis has been accumulated,[1] but little has been published concerning the experimental approach to other types of lipoidosis. Pasternack and Page[2] found no change in the phosphatide content of the brain following repeated intravenous injections of cephalin. No histologic examination of the brain was made. Sjövall,[3] working with intraperitoneal and intravenous injections of lecithin, failed to obtain significant pathologic changes in rabbits. Kimmerstiel and Laas,[4] using mice, injected cerebrosides and lecithin intraperitoneally and observed a local reaction which consisted of numerous foam cells when cerebrosides were injected, while histiocytes and epithelial cells made up the local reaction to injections of lecithin. Moreover, with intravenous injections of cerebrosides in rabbits these investigators observed infiltration of the spleen and liver with foamy cells similar in structure

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This investigation was aided by a grant from Child Neurology Research (Friedsam Foundation).

1. For a complete review of the literature concerning experimental cholesterinosis see Duff, G. L.: Arch. Path. **20**:81, 1935.

2. Pasternack, L., and Page, I. H.: Biochem. Ztschr. **252**:254, 1932.

3. Sjövall, A.: Acta path. et microbiol. Scandinav. **12**:307, 1935.

4. Kimmerstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**: 417, 1934.

to those observed in Gaucher's disease. As far as the spleen is concerned, these results were recently confirmed by Klenk and Goebel,[5] who, in addition, obtained a similar pathologic condition with injections of cerebron.

Finally, Beumer and Gruber[6] reported having found foam cells in the spleens and livers of 2 rabbits given intravenously 6 and 7 Gm. of sphingomyelin, respectively; in a third rabbit, which received 9 Gm. of this lipoid, there were, in addition, slight changes in the kidney and the bone marrow. Negative results were obtained with injections of the same amount of a mixture of lecithin and cephalin.

In this paper, experimental lipoidosis due to phosphatides will be considered. The purpose of the experiments was twofold: (1) to attempt experimental reproduction of the pathologic changes of Niemann-Pick disease with the view of contributing to the study of its genesis; (2) to approach experimentally the problem of the relationship between Niemann-Pick and Tay-Sachs disease in an attempt to establish whether these two conditions are the expression of two different localizations of the same disordered lipoid metabolism (Bielschowsky;[7] Spielmeyer[8]) or are entirely different diseases (Schaffer;[9] Epstein[10]).

EXPERIMENTAL PROCEDURES

Since evidence has been recently presented by Klenk[11] and confirmed by others[12] that the phosphatide infiltrating the spleen and liver in Niemann-Pick disease is sphingomyelin, this lipoid was chosen in the attempt to reproduce the condition in animals.

Extraction of Sphingomyelin.—For the preparation of sphingomyelin, the method of Klenk[11] slightly modified was used by one of us (G. A. J.). Calf brains were thoroughly passed through a meat grinder. The material was then extracted with 4 volumes of acetone at room temperature for twenty-four hours. The acetone treatment was repeated five times with fresh solvent. The organ powder was subsequently extracted four times with ether at room temperature. The air-dried powder was suspended in several volumes of a mixture of chloroform and methyl alcohol (methanol), 1:3, and kept at boiling temperature for thirty minutes. This treatment was repeated twice. The hot solution was then filtered and evaporated to dryness. The main part of crude sphingomyelin was found

5. Klenk, E., and Goebel, A.: Deutsche Ztschr. f. Verdauungs- u. Stoffwechselkr. **1**:151, 1938.

6. Beumer, H., and Gruber, G. B.: Jahrb. f. Kinderh. **146**:126, 1936.

7. Bielschowsky, M.: J. f. Psychol. u. Neurol. **36**:103, 1928.

8. Spielmeyer, W.: Klin. Wchnschr. **12**:1273, 1933.

9. Schaffer, K.: Ztschr. f. Neurol. u. Psychiat. **139**:790, 1932.

10. Epstein, E.: Virchows Arch. f. path. Anat. **293**:134, 1934.

11. Klenk, E.: Ztschr. f. physiol. Chem. (*a*) **229**:151, 1934; (*b*) **235**:24, 1935.

12. Tropp, C., and Eckardt, B.: Ztschr. f. physiol. Chem. **243**:39, 1936. Chargaff, E.: J. Biol. Chem. **130**:503, 1939.

in the last mixture. However, a certain amount was extracted by ether, from which it could be recovered by precipitation after standing twenty-four hours at 0 C.

For further purification, the crude combined product was washed with acetone and dissolved in ten volumes of hot methyl alcohol. Impurities were precipitated off by adding a small amount of a concentrated solution of cadmium acetate in methyl alcohol. The hot solution was filtered again, and the sphingomyelin obtained after evaporation of the methanol was extracted with acetic acid and successively recrystallized from ethyl acetate and pyridine. The yield was very small. The purified product was a whitish powder insoluble in ether and acetone and soluble in warm methyl alcohol. The melting point was between 200 and 210 C. The nitrogen-phosphorus ratio varied between 1.9 and 1.98. The Molisch reaction was still weakly positive, indicating the presence of small amounts of cerebrosides. Further purification was attempted by using ethyl alcohol to precipitate off the cerebrosides from a solution of the sphingomyelin in a mixture of benzene and alcohol (5:1), following the directions of Levene.[13]

Injections of Sphingomyelin.—Two monkeys (Macacus rhesus) and 11 rabbits were used. The lipoid was injected intravenously as a fine emulsion, made by adding, drop by drop, to boiling water a solution of sphingomyelin in hot alcohol. One monkey died after two injections and was discarded since post mortem there were diffuse and severe tuberculous lesions. Three rabbits died following a few injections, presumably of embolism; they also were discarded. In the other rabbits the total dose varied from 8 to 30 Gm. Groups of 2 rabbits each received, respectively, 8, 12, 20 and 30 Gm., and 1 monkey, 21 Gm. The injections were given almost daily. The single dose varied from 0.25 to 1.5 Gm. All the animals were killed with ether and immediately examined.

Histologic Technic.—The following stains were used in the study of the involved tissues: hematoxylin-eosin, Masson's trichrome and Mallory's aniline blue. Various methods were used for demonstrating lipoid substances. In addition, the nervous system was investigated with the usual procedures of neuropathologic technic, including the Nissl, Spielmeyer, Bielschowsky, Cajal and Hortega methods.

PATHOLOGIC CHANGES

Since the pathologic features were almost identical in all the animals, a general description of them will be made.

Gross examination of the viscera showed, first, considerable enlargement of the spleen, which in rabbits that received 30 Gm. of lipoid appeared over four times the normal size. The splenic tissue was dry and hard. The liver was also enlarged in many instances, although to a much less degree than the spleen. Occasionally the lung showed areas of apparent hepatization. The marrow showed a yellowish instead of the normal red color. In 2 rabbits the adrenal glands appeared somewhat enlarged. The other organs and tissues were grossly normal.

The fundamental histologic lesion consisted of the presence in the affected organs of characteristic large round or polyhedral cells. In the hematoxylin-eosin preparation these cells showed abundant cytoplasm, which exhibited a foamy or honeycomb structure, represented by numerous small vacuoles. In some cells the small vacuoles had apparently coalesced, thus forming one or two large vacuoles, which occupied the whole cytoplasm. In frozen sections cleared with cedarwood

13. Levene, P. A.: J. Biol. Chem. **18**:453, 1914.

oil and mounted in glycerin the small vacuoles appeared filled with a material that stained light red with eosin, purple-red with carmine, pale rose with sudan III and scarlet red, bluish brown with hematoxylin, yellow with safranin, blue with aniline blue and lavendar with nile blue; it did not reduce osmic acid. The

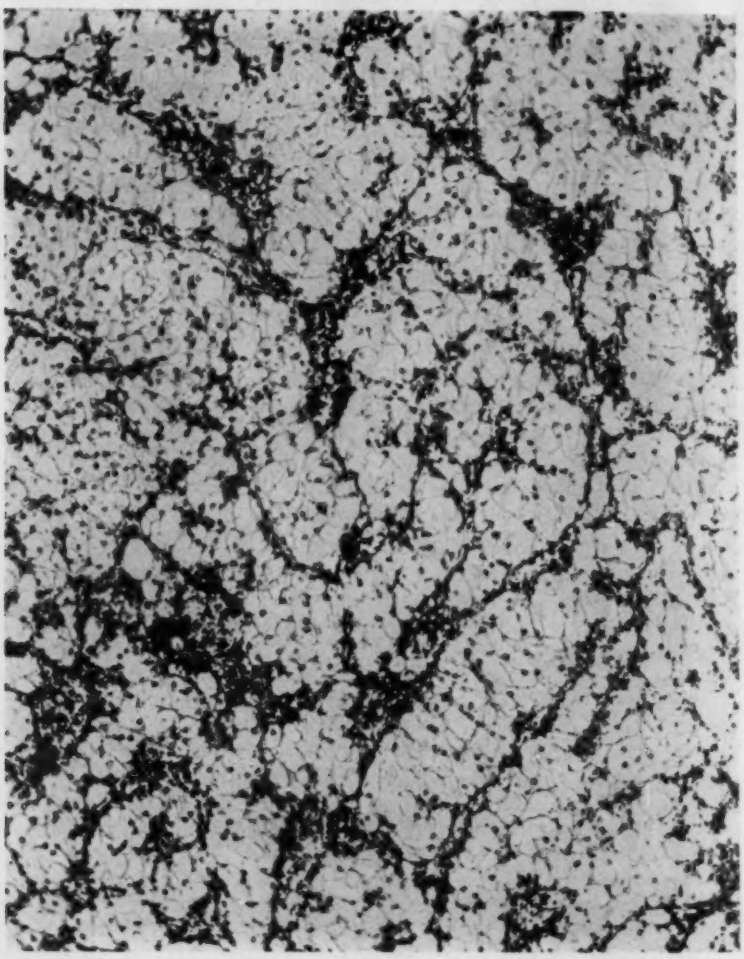

Fig. 1.—Section of a spleen showing the transformation of the splenic pulp into a mass of foamy cells (Mallory's trichrome stain).

material was not dissolved by cold acetone, cold ether and cold alcohol but was dissolved by hot alcohol or by a mixture of chloroform and methyl alcohol or by xylene or by benzene.

The foam cells generally contained one nucleus, which was located toward the periphery; occasionally two nuclei were present, and exceptionally three were

observed. Syncytia were apparently absent. The nucleus was generally small and round; it often appeared shrunken, darkly stained and without a nucleolus. Nuclear mitoses were never observed.

Altogether, in their morphologic characteristics these foam cells bore striking similarities to the so-called Pick's cells found in human material.

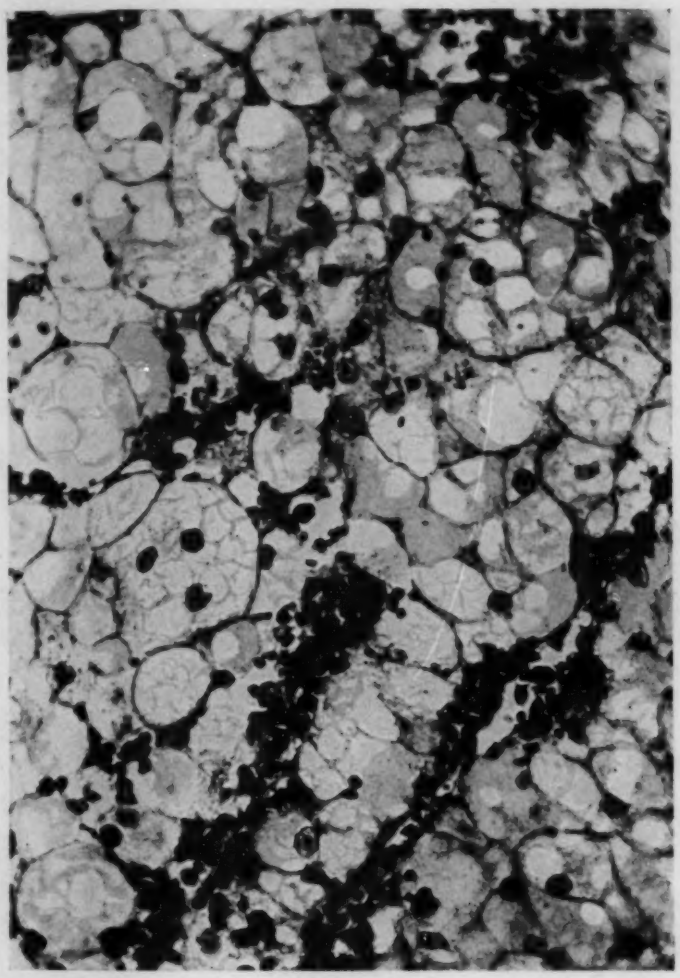

Fig. 2.—Section of a spleen showing, at high magnification, the characteristics of the foamy cells (Mallory's trichrome stain).

Spleen.—The splenic pulp was no longer recognizable, having been transformed into a mass of foamy cells (figs. 1 and 2), which filled the spaces between the sinuses. The sinuses were considerably narrower than those of a normal spleen and consequently contained a much less amount of blood, but they stood out

prominently against the background of pale cells. In many fields the sinus walls were apparently normal, consisting of elongated cells whose cytoplasm contained no lipoids. In other instances it was difficult to decide whether the endothelial cells of the sinus wall had also undergone transformation into foam cells. Occasionally, a large Pick's cell was found to lie free in the lumen of a sinus.

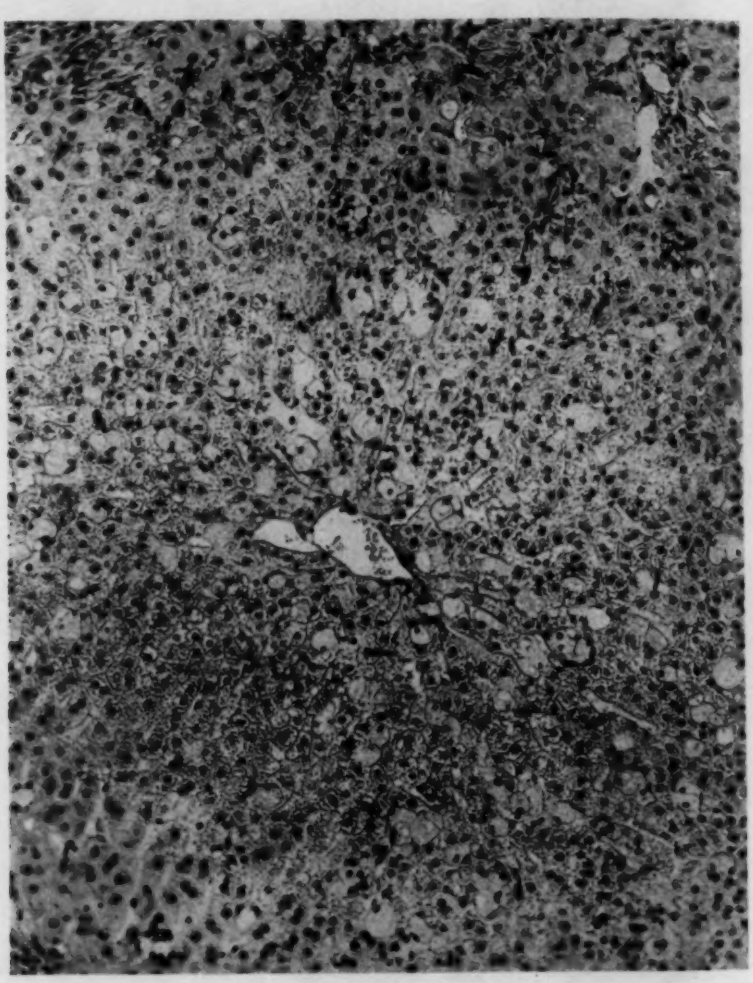

Fig. 3.—Section of a liver lobule showing numerous foam cells (hematoxylin-eosin stain).

The malpighian bodies were still present, although their size was smaller than normal. There was often a group of foam cells within the follicles. No significant increase of connective tissue was present. Hemorrhages were never observed. Sections of spleen did not react when Turnbull's blue stain was applied for hemosiderin.

These pathologic alterations of the spleen were constant in all the animals that received 20 or 30 Gm. of sphingomyelin. Lesions similar in character were found in the others, but the pathologic process was not so severe, the malpighian bodies being generally well preserved and several normal splenic cells present among the foam cells.

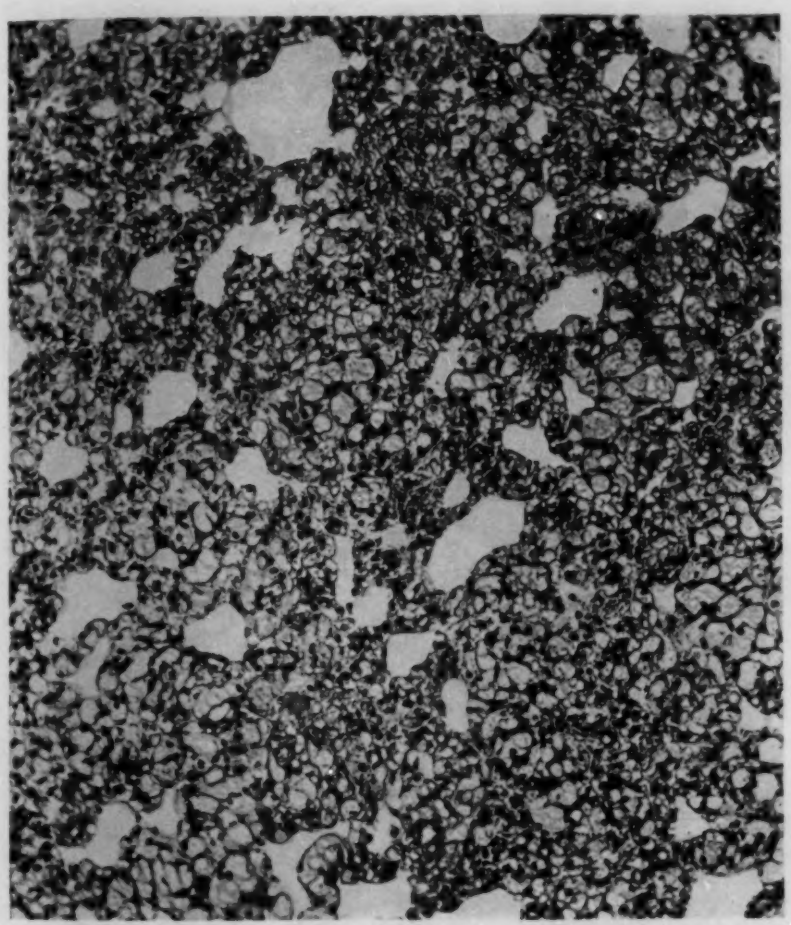

Fig. 4.—Section of a lung showing infiltration of the parenchyma by foam cells (Mallory's trichrome stain).

Liver.—In the animals receiving 20 or 30 Gm. of lipoid the normal architecture of the hepatic lobules was considerably modified (fig. 3), the rows of liver cells being interrupted by a large number of foam cells, which apparently originated from the sinusoids. These foam cells could be differentiated from the parenchymal cells, being larger, paler, with small eccentric nuclei and vacuolar cytoplasm. In Mallory's aniline blue preparation the liver cells were stained orange or orange-

red, while the foam cells were blue. In some fields foam cells outnumbered liver cells. The sinusoids contained very little blood, and only a few normal Kupffer cells could be recognized. In the Mallory preparation it was sometimes possible to trace in the same section apparently transitional phases between normal Kupffer cells and large foam cells. The intermediate stages consisted of enlarged Kupffer

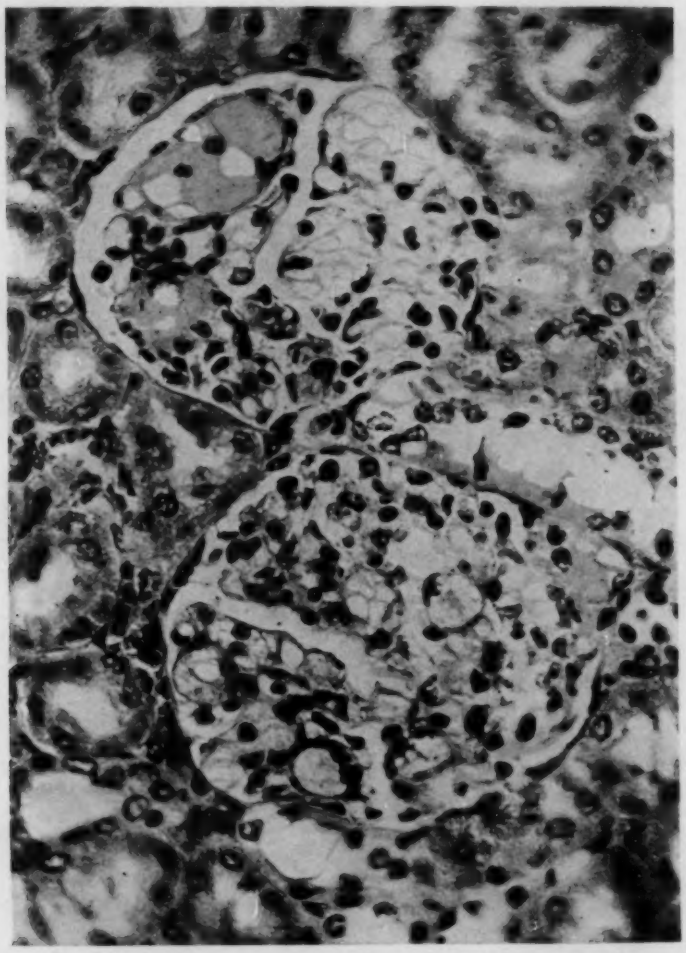

Fig. 5.—Section of a kidney showing foam cells within two glomeruli (hematoxylin-eosin stain).

cells containing a few blue granules. Foam cells were present also in the connective trabeculae. In the periportal connective tissue there were occasionally found small groups of plasma cells and lymphocytes. There was no cirrhosis.

In the animals that received 30 Gm. of sphingomyelin, granules exhibiting the staining properties of the lipoid content of the foam cells were present also within

the cytoplasm of the parenchymal cells. In an animal that received 8 Gm. of lipoid, no foam cells were found in the liver.

Lungs.—Marked infiltration of the lungs with foam cells was constantly observed in the animals that were given 20 and 30 Gm. of lipoid. In the most advanced stages it was difficult to identify the pulmonary alveoli, the whole

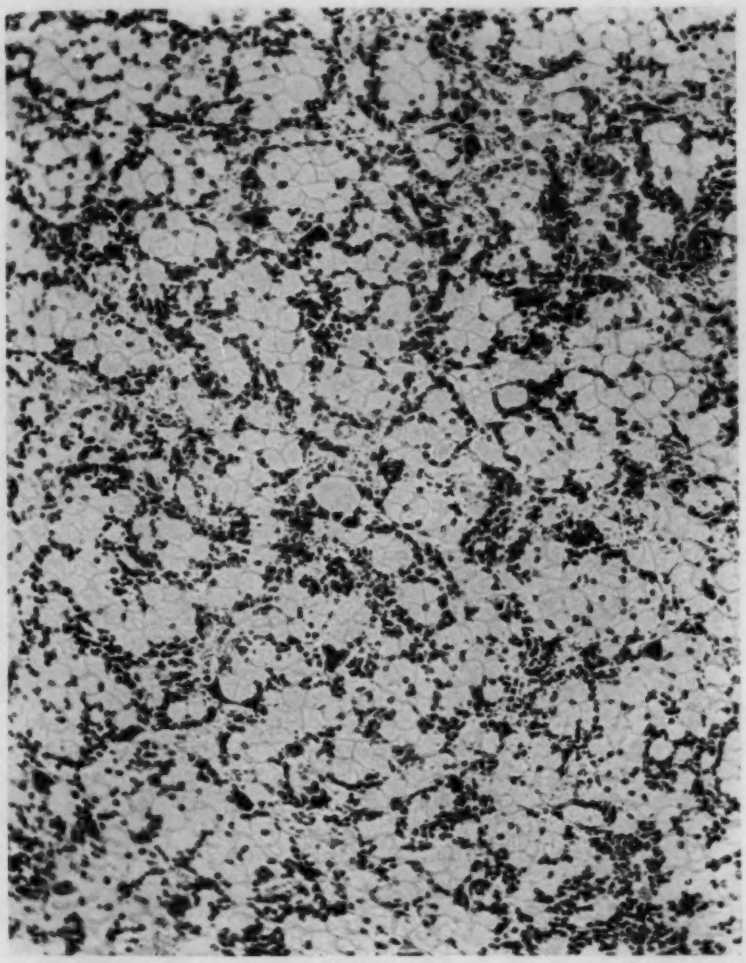

Fig. 6.—Section of bone marrow showing the transformation of the tissue into a mass of foam cells (Mallory's trichrome stain).

parenchyma having been transformed into a mass of foamy cells, in which only the connective tissue could be differentiated. In other regions the alveoli could still be recognized, but the alveolar spaces were filled with foam cells (fig. 4). The alveolar epithelium had often disappeared. It appears possible that the foam cells derived partly from the alveolar epithelium, although no transitional form

could be definitely recognized. Pick's cells infiltrated also the interlobar and peribronchial connective tissue. The bronchial epithelium showed no infiltration.

Kidneys.—Large foamy cells were found in many of the glomeruli (fig. 5). At times a whole glomerulus had been transformed into a mass of Pick's cells, the glomerular loop being no longer recognizable; at other times a few large cells occupied the center of a glomerulus, displacing to the periphery a part of the vascular loop. The space between the glomerular loop and the Bowman membrane was generally free. This membrane apparently was not active in the formation of scavenger cells. In some sections there was hardly a glomerulus which did not contain foam cells. Pick's cells were not found in other parts of the kidney.

Adrenal Glands.—These organs also were almost constantly involved. The cortex contained large foam cells, the size of which was twice that of the cells of the epithelium proper. The lipoid content of the foam cells was easily differentiated from the lipoid of the gland, the latter being stained bright red with scarlet red. The medullary portion of the adrenal also contained foam cells, although in a lesser number.

Bone Marrow.—The fat tissue had almost completely disappeared in the regions most involved and was replaced by typical foam cells, which in numerous instances largely outnumbered the cellular elements of the blood-forming tissue (fig. 6). Repeated examination of blood preparations failed to detect Pick's cells or swollen histiocytes free in the blood stream.

Brain.—Particular attention was paid to the brain. Nissl preparations of the representative regions showed no alteration of the normal morphologic pattern of the neuron cell. Increase of size, disappearance of the Nissl bodies, accumulation of lipoids in the cytoplasm and displacement of the nucleus, all features characteristic of the Nissl picture in amaurotic idiocy, were never seen. Specific stains failed to detect lipoids within the neuron cell. The myelin was likewise entirely normal.

Cajal's preparations for neuroglia showed no significant changes, nor was fat found within the neuroglial cytoplasm. Hortega's method for microglia revealed some hypertrophic changes in 2 cases. The microgliacytes were larger than normal; their cytoplasm was swollen, and the dendrites were irregular in shape, showing small circumscribed swellings. In preparations counterstained by methods specific for fat, no lipoid substance was found in the swollen cytoplasm.

The blood vessels of the brain were normal; their endothelium nowhere showed transformation into foam cells.

COMMENT

The question as to the origin of the foam cells in lipoidosis has been generally answered by assuming that the histiocytes are responsible for the storage of lipoid. This assumption appears to be confirmed by the present experiments. Thus, the foam cells of the spleen and the bone marrow presumably originated from the reticulum, those of the liver from the Kupffer cells, and the Pick's cells in the adrenal from the capillaries, since it is known that some of the cells of the walls of the venous capillaries of the adrenals belong to the histiocytic system. More difficult was it to interpret the source of the foam cells of the alveolar epithelium. However, if one accepts the view of Lang [14] concerning

14. Lang, F. J.: J. Infect. Dis. **37**:430, 1925.

the histiocytic nature of the so-called alveolar phagocytes of the lung, one must admit that it was from these histiocytes rather than from the epithelial cells that Pick's cells in our cases had derived.

It will be noted that the changes did not involve uniformly the entire histiocytic system. In fact, the cells lining the sinuses of the spleen, while storing vital dyes, apparently failed to phagocytose sphingomyelin. Such an observation, however, has been repeatedly made in human cases of lipoidosis. On the other hand, in 1 case, at least, the lipoid storage was present in the parenchymal cells of the liver outside the histiocytic system.

That the histiocytes are mainly responsible for the formation of the foam cells was evidenced by a further experiment: Six grams of sphingomyelin was injected into a rabbit in which so-called blockage of the reticuloendothelial system had been accomplished by means of repeated injection of congo red. In this animal only a few Pick's cells were seen, together with the well known picture of dye storage. At this point it seems of interest to stress the difference between the reaction of histiocytes to sphingomyelin and their reaction to colloidal stains. In the latter the histiocytes are swollen but maintain their normal number and distribution, being simply laden with the foreign bodies; in the former they apparently increase in size and number and infiltrate the tissue proper of the organ, disturbing its morphologic aspects and presumably its functional efficiency.

Whether the tendency to form foamy cells depends on the chemical constitution of the lipoid injected or on its colloidal character [4] is difficult to decide. It will be noted, however, that injections of lecithin produce foam cells only temporarily (Sjövall [3]), and cephalin fails to give any reaction (Beumer and Gruber [6]). The last report was confirmed by our findings in 2 rabbits which were given 10 Gm. of cephalin (prepared according to the method of Levene [15]); no foam cells were seen in the spleen and liver. As far as phosphatides are concerned, therefore, only sphingomyelin appears to have this specific action on the histiocytic system. It will be noted that sphingomyelin is the only phosphatide thus far found to be present in human lipoidosis.

There seems to be little doubt that the pathologic changes here described simulate closely those found in human Niemann-Pick disease. The structural and tinctorial characteristics of the foam cells are the same, and their wide distribution in the spleen, liver, lungs, marrow, kidney and adrenal bodies corresponds to what has been repeatedly described in man. According to Bloom,[16] in Niemann-Pick disease the

15. Levene, P. A., and Rolf, J. P.: J. Biol. Chem. **74**:713, 1927.

16. Bloom, W.: Am. J. Path. **1**:595, 1925.

lipoid storage is limited to the histiocytic system; hence, the term "lipoid histiocytosis." However, other investigators (Pick;[17] Baumann[18]) have reported storage of lipoid in the parenchymal cells of various organs. It is reasonable to assume that the latter type of storage occurs only in advanced stages of the disease. In fact, in the rabbits which were given high doses of sphingomyelin lipoid granules were present in the liver cells.

Our experiments may throw some light on the problem of genesis of Niemann-Pick disease. The hypothesis of Pick[17] that a primary disorder of the metabolism of phosphatides is at the basis of the disease appears consistent with the findings here reported. It seems, in fact, reasonable to assume that in man this metabolic disorder leads to an overloading of the organism with sphingomyelin, resulting in a reaction similar in its morphologic character to that caused by injection of sphingomyelin. According to this hypothesis, Niemann-Pick disease should be included in that group of diseases characterized by an error of metabolism consisting in a stoppage of a certain metabolism at a certain intermediate stage. In the specific instance of Niemann-Pick disease the fat metabolism, the cycle of which probably includes a stage of phosphatides,[19] is altered. It is also possible that the normal choline metabolism is especially involved, since sphingomyelin is formed by choline.[20] Whether the presence of an abnormally high amount of sphingomyelin is due to a defect in catabolism caused by a primary alteration of cellular phosphatases as postulated by Sobotka[21] or to an increase in anabolism of phosphatides caused by hyperadrenalism as maintained by Epstein[22] remains open to investigation. Thus far neither hypothesis has the support of convincing evidence.

Concerning the second purpose of this investigation, viz., the experimental approach to the problem of the relationship of Niemann-Pick disease to Tay-Sachs disease, little evidence was obtained.

It is well known that the study of amaurotic idiocy has received a new impulse since the discovery that pathologic features characteristic of Niemann-Pick disease are frequently found in Tay-Sachs disease.[23]

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17. Pick, L.: Ergebn. d. inn. Med. u. Kinderh. **29**:520, 1926.

18. Baumann, T.; Klenk, E., and Scheidegger, S.: Ergebn. d. allg. Path. u. path. Anat. **30**:183, 1936.

19. Sinclair, R. G.: J. Biol. Chem. **115**:211, 1936.

20. Best, C. H., and Ridout, J. H.: Choline as a Dietary Factor, in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1939, vol. 8, p. 349.

21. Sobotka, H.; Glick, D.; Reiner, M., and Tuchman, L.: Biochem. J. **27**: 2031, 1933.

22. Epstein, E.: Ergebn. d. allg. Path. u. path. Anat. **33**:280, 1937.

23. Bielschowsky.[7] Spielmeyer.[8]

Consequently, the opinion has been repeatedly expressed that the two conditions are manifestations of the same disorder of fat metabolism. For a clearer understanding of this problem it may be of assistance to distinguish three groups of cases, namely, (1) cases of Niemann-Pick disease without brain involvement of the Tay-Sachs type, (2) cases of Tay-Sachs disease without visceral involvement of the Niemann-Pick type and (3) cases in which features of the two diseases coexist. The question is whether one is dealing with the same disease or with two or possibly three different conditions. The experiments reported here were planned in the hope of clarifying this question. It was thought that a study of the brain would offer some evidence indicating whether fundamental histologic differences occur between "experimental Niemann-Pick disease" and amaurotic idiocy. Unfortunately, the attempt to produce storage of sphingomyelin in the nervous system failed. Doses which caused widespread lesions in almost every other organ produced no change in the brain. Even the microglia, which is generally considered as part of the reticuloendothelial system, showed no scavenging of lipoid, although in 2 instances hypertrophic changes of microgliacytes could be observed. It appears, therefore, that under experimental conditions the brain offers considerable resistance to accumulation of phosphatides. Whether increased permeability of the hematoencephalic barrier can lessen this resistance will be indicated by new experiments, now in progress, in which the lipoid is injected following a lesion of this barrier.

On this basis of the little evidence obtained in the present experiments one may attempt to explain the nature of the three groups of cases just referred to. In the first group the disease could be understood as caused by a metabolic disorder affecting sphingomyelin. Moreover, it might be possible that in advanced Niemann-Pick disease the lipoid storage will involve also the parenchyma, including the neuron cells; there would then result a brain picture closely allied to that of Tay-Sachs disease and coexistent with the features of Niemann-Pick disease (group 3). However, as far as cases of Tay-Sachs disease without visceral involvement are concerned (group 2), it appears difficult to conceive of instances of general metabolic alteration of sphingomyelin in which the lipoid is stored exclusively in the brain, an organ which offers particular resistance to the storage of sphingomyelin. It is more reasonable to assume that in these cases a different process is at the basis of the condition. Hence, a difference might exist between Niemann-Pick and pure Tay-Sachs disease.

This assumption seems supported by recent data in the literature [24] indicating that an abnormal amount of sphingomyelin is not present in

24. Klenk, E.: Ber. ü. d. ges. Physiol. u. exper. Pharmakol. **96**:659, 1936.

the brain in cases of pure Tay-Sachs disease (group 2) while it is present in cases in which features of Tay-Sachs and Niemann-Pick disease coexist (group 3).[11b]

SUMMARY

Experiments are reported in which rabbits and monkeys were given high doses of sphingomyelin intravenously. Morphologically, the resulting changes bore striking similarities to the pathologic picture of human cases of Niemann-Pick disease. The relation of Niemann-Pick to Tay-Sachs disease is discussed. Although the data presented offer only indirect evidence, the view is expressed that Tay-Sachs disease when occurring without hepatosplenic involvement represents presumably a condition the genesis of which differs from that of Niemann-Pick disease.

CORRECTION

In the article by Dr. G. A. C. Snyder entitled "Spontaneous Double Rupture of the Heart," in the June issue (ARCH. PATH. **29**:796, 1940), the word "arteriosclerosis" in lines 8 and 14 on page 799 should read "arteriolosclerosis."

Case Reports

LEFT RETROMESOCOLIC HERNIA

Three Additional Cases

BÉLA HALPERT, M.D., NEW ORLEANS

In a recent report [1] on a left retromesocolic hernia in a colored man 55 years of age, the mode of development of this type of hernia was discussed. Three additional examples of left retromesocolic hernia have since been observed in the necropsy material of the Charity Hospital of Louisiana at New Orleans. The cases are recorded here because certain features are presented which further contribute to the understanding of this condition.

In each of these cases, as in the cases of the right [2] and the left retromesocolic hernia previously reported, the hernias produced no clinical signs or symptoms and were discovered at necropsy. For this reason details of the clinical histories are omitted, and only the pertinent findings at necropsy are presented.

REPORT OF CASES

CASE 1.—A colored man 59 years of age was admitted to the hospital, Dec. 21, 1938, following a "stroke" twenty-four hours before. He was unconscious on admission and died Jan. 6, 1939.

The necropsy revealed the following conditions: cardiac hypertrophy and dilatation; sclerosis of the renal and cerebral arteries; scarring and atrophy of the kidneys, with left renal infarct; infarct in the left lenticular nucleus; passive congestion of the viscera; bronchitis and bronchiolitis with bilateral focal pneumonia; decubitus ulcer of the sacrum; hyperplasia of the prostate; fibrous pleural adhesions on the right side, with calcification; left retromesocolic hernia.

The peritoneal cavity contained no excess of fluid. Its surfaces were smooth and glistening. The inferior margin of the liver was 3 cm. above the costal margin in the right midclavicular line. The diaphragm was at the level of the fourth interspace on both sides. The appendix, which was in a medial position, was 7 cm. long. The cecum, colon and rectum revealed no abnormalities. The mesenteric lymph nodes were not conspicuous.

Below the transverse mesocolon, in the region of the duodenojejunal fossa, was a peritoneal pocket with an opening 12 by 6 cm. and 8 cm. deep, which contained about 100 cm. of intact jejunum together with the left inframesocolic portion of the duodenum. Near the free margin of the anterior wall of the peritoneal pocket the inferior mesenteric vein and the ascending branch of the left colic artery ran in their usual relationship.

CASE 2.—This case has been reported in full elsewhere.[3] A colored woman 26 years of age, the mother of 3 children, was admitted to the hospital, April 5,

From the departments of pathology and bacteriology of Charity Hospital of Louisiana at New Orleans and the Louisiana State University School of Medicine.

1. Halpert, B.: Surgery **5**:379, 1939.
2. Halpert, B.: Surgery **3**:579, 1938.
3. Schattenberg, H. J., and Ziskind, J.: South. Surgeon, to be published.

1939, twenty-four hours after delivery of a normal, full term child. The placenta was still in situ. The patient was acutely ill on admission and did not respond to conservative measures. Dilatation and curettage were performed nine days after admission. Death occurred April 25, 1939.

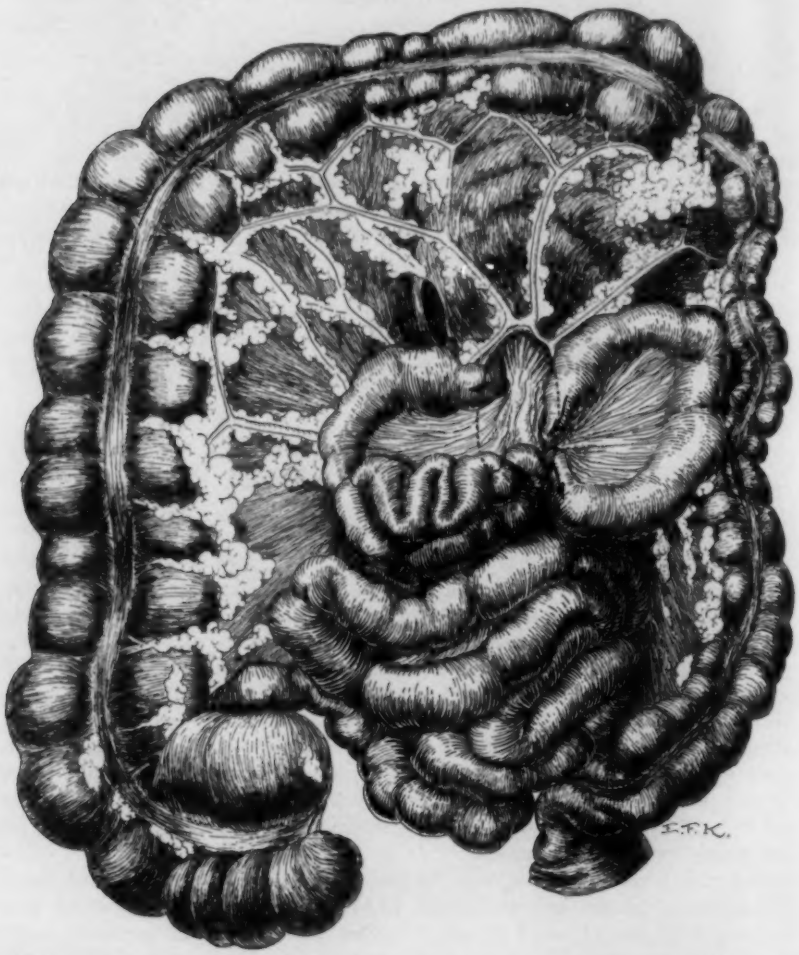

Fig. 1.—Left retromesocolic hernia in a Negro boy 7 years of age. Three loops of small intestine emerge from an opening on the left side of the vertebral column in the region of the duodenojejunal fossa. This opening, which is 4 by 3 cm., leads into a pocket, 8 by 8 cm., which contains the left inframesocolic portion of the duodenum and 20 cm. of the contiguous jejunum. The jejunum leaves the pocket through the distolateral portion of the opening, forms an exterior loop 20 cm. long and returns to the pocket through the craniolateral portion. It remains within the pocket for 50 cm. and leaves through the craniomedial portion to continue into the distal jejunum and ileum.

The necropsy (Dr. Joseph Ziskind) revealed the following conditions: acute endometritis, post partum, with septicemia; multiple abscesses in the lungs and the right kidney; enlargement of the spleen; left retromesocolic hernia.

The peritoneal cavity contained no free fluid. Its surfaces were smooth and glistening. The inferior margin of the liver extended 4 cm. below the xiphoid process. The diaphragm was at the level of the fifth rib on the right and the sixth rib on the left side. The loops of intestine were somewhat distended with gas. The cecum, colon and rectum revealed no abnormalities. The mesenteric lymph nodes were conspicuous.

Below the transverse mesocolon, in the region of the duodenojejunal fossa, was a peritoneal pocket with an opening 10 by 8 cm. and 7 cm. deep, which contained about 30 cm. of jejunum together with the left inframesocolic portion

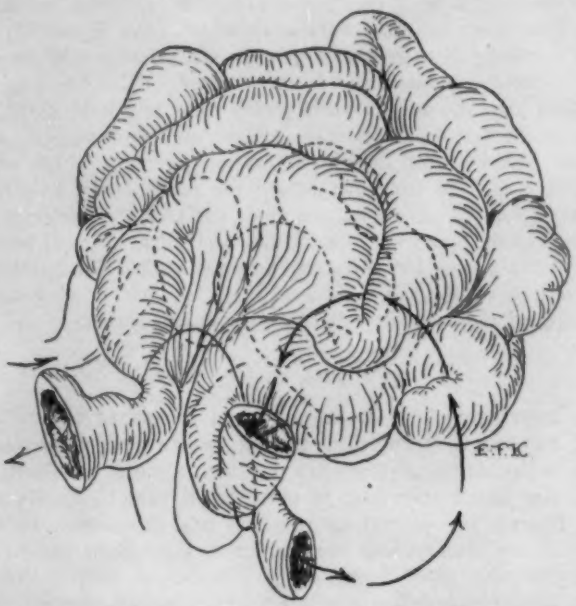

Fig. 2.—Position of the loops of jejunum contained in the peritoneal pocket. The dotted line indicates the duodenojejunal junction and the 20 cm. of contiguous jejunum. The jejunum leaves the pocket through the distolateral portion of the opening, forms an exterior loop, indicated by arrows, then returns into the pocket for a length of 50 cm. It leaves through the medial portion of the opening to continue into the distal jejunum and ileum.

of the duodenum. The loops of jejunum in the sac were free of adhesions and easily removed. The inferior mesenteric vein and the ascending branch of the left colic artery ran in their usual relationship in the anterior wall of the sac near its medial border.

Case 3.—A Negro boy 7 years of age was admitted to the hospital Aug. 10, 1939. He had been hydrocephalic from birth. He had had a persistent headache for ten weeks and had lost the sight of both eyes fourteen days before admission. Exploratory craniotomy was performed September 8, but no lesion was located. The boy died Oct. 6, 1939.

The necropsy (Dr. Edward L. Burns) revealed: a craniotomy wound in the occipital region; glioma of the left parietal and occipital lobes; acute purulent leptomeningitis; bilateral renal calculi; bilateral hydronephrosis; left retromesocolic hernia.

The peritoneal cavity contained no excess of fluid. Its surfaces were smooth and glistening. The inferior margin of the liver was at the costal margin in the right midclavicular line. The greater omentum was delicate and free of adhesions. No changes were noted in the relation of the organs in the upper part of the abdomen. The appendix, which was 7 cm. long, was in the medial position. The cecum was mobile. The mesenteric lymph nodes were not conspicuous.

In the transverse mesocolon, near the root, was a longitudinal slit, 2 by 0.5 cm. Below the transverse mesocolon, in the region of the duodenojejunal fossa, was an oval opening, 4 by 3 cm. From this opening, which led into a pocket, 8 by 8 cm., three loops of small intestine emerged (figs. 1 and 2). The mediocranial loop continued into the distal part of the jejunum and ileum. The distolateral loop continued exteriorly for a distance of 20 cm. and returned as the craniolateral loop into the peritoneal pocket for a length of 50 cm. The pocket thus contained the left inframesocolic portion of the duodenum, with 20 cm. of contiguous jejunum, and another 50 cm. of jejunum, which returned after having formed a loop 20 cm. long outside the pocket. The loops contained in the pocket were free of adhesions, and their surfaces were smooth and glistening. The lumen of the bowel was slightly narrowed at the point of passage through the opening of the pocket, but there was no obstruction. The inferior mesenteric vein and a branch of the left colic artery ran in their usual relationship near the free margin of the anterior wall of the peritoneal pocket.

COMMENT

As has been previously pointed out, a hernia of this type is to be interpreted not as a herniation into a preformed peritoneal sac but rather as a malposition of the part of the intestine involved. The malposition of the small intestine is considered due to faulty orientation of the gut during the period of rotation and to failure of the craniomedial half of the descending mesocolon to fuse with the parietal peritoneum. Normally, after rotation of the bowel and fusion with the peritoneum are completed, a slight pocket remains, the duodenojejunal fossa. In cases with this malposition of the small intestine, however, a large pocket is formed which encloses part of the duodenum together with some loops of contiguous jejunum. Since the descending mesocolon forms the anterior wall of this pocket, the condition is appropriately termed left retromesocolic hernia.

Although the patients were of different ages (a boy of 7, a woman of 26 and a man of 59), in each of them the peritoneal sac was at the same site and of almost identical size; each sac contained varying lengths of the initial portions of the small intestine, and the vascular relations in the anterior wall of the sac were those of the descending mesocolon. These facts favor the concept that this condition is not acquired but develops before the rotation of the gut and fusion of the peritoneum are completed.

SUMMARY

Three cases of left retromesocolic hernia are recorded, in all of which the abnormality was an incidental observation at necropsy. The

condition is interpreted as a malposition of a segment of the small intestine behind the descending mesocolon. It is regarded as due to faulty orientation of the gut during its rotation and to failure of the craniomedial half of the descending mesocolon to fuse with the parietal peritoneum. A hernial sac is thus formed, the anterior wall of which contains the inferior mesenteric vein and the left colic artery in their usual relationship and which is a part of the descending mesocolon, hence the name "hernia retromesocolica sinistra."

LEIOMYOSARCOMA OF THE PLEURA

A Case with Metastases

WALTER A. STRYKER, CHICAGO

In a report in 1935 on the incidence of malignant tumors arising from smooth muscle McFarland [1] was able to cite only 1 instance of leiomyosarcoma primary in the pleura. This was the case reported in 1931 by Catron,[2] in which a tumor mass, 22 by 18 by 8 cm., was found in the left pleural cavity of an 83 year old woman; microscopically this tumor was diagnosed as leiomyosarcoma although no fibrils were visible.[3] No metastases were found; the tumor was described as incidental, with death due to severe sclerosis of the coronary arteries. In 1938 Jaffé [4] reported 2 cases of fibromyoma of the pleura with what were interpreted as metastases in regional lymph nodes, although on histologic examination the primary lesions were considered benign.

REPORT OF CASE

An 18 year old white school girl was admitted to the University of Chicago Clinics, Sept. 16, 1937, to the service for patients with diseases of the chest. Three months previously an acute pain developed in the left side of the chest; this disappeared after one week of rest in bed but recurred two weeks later. The pain was accompanied by a productive cough; the sputum, however, contained no tubercle bacilli nor were bacilli found in a small amount of reddish fluid aspirated from the the left side of the chest. During the subsequent course of the illness pain in the chest remained severe and nearly constant. There was a daily elevation of temperature, up to 103 F. There was no dyspnea, but palpitation was occasionally marked, and the patient had the sensation that her heart was pushed over to the right side. A swelling of both arms had been noted for several weeks, and the veins on the thoracic wall were dilated. She had lost 25 pounds (11 Kg.) since the onset of the illness. The past history was noncontributory except that one year previous to examination the patient had noted a curvature of the spine; this had not increased during the year.

At the time of her admission the prominence of the left anterior thoracic wall was observed. The veins on the anterior surface of the chest were dilated and tortuous. Expansion of the left side of the chest was limited. There was flatness of the left side of the thorax to percussion; breath and voice sounds

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From the Department of Pathology, University of Chicago.

1. McFarland, J.: Am. J. Cancer **25**:530, 1935.

2. Catron, L.: Arch. Path. **11**:847, 1931.

3. "The cytoplasm was in most parts fusiform, extending from the two nuclear poles as homogeneous bands, but in some places it formed fine processes extending in various directions from the nucleus. Some of the cell tips were branched. No fibrils were visible in these cells, and the Bielschowsky silver impregnation revealed no intervening reticulum."

4. Jaffé, R. H.: Arch. Path. **25**:60, 1938.

were absent. The heart was displaced far to the right. The patient was emaciated; there were numerous areas of brown pigmentation over the entire body. On one occasion the right pupil was noted to be slightly larger than the left. Roentgenologic examination revealed a neoplasm involving the upper lobe of the left lung, with invasion of the bodies of the third and fourth thoracic vertebrae and probable invasion of the second, third and fourth ribs at their vertebral ends. The blood leukocyte count was 41,000, with 91 per cent polymorphonuclear leukocytes. The blood pressure in the dilated right external jugular vein was 280 mm. of water. The patient's course in the hospital was rapidly downhill; thoracentesis was attempted several times without success; the fever continued. Two roentgen ray

Fig. 1.—Anterior view of the tumor, showing marked displacement of the heart. Rib markings are seen on the neoplasm.

treatments of 219 roentgens each were given over the left thoracic portals, September 21 and 22. Sedatives were required constantly; the white blood cell count steadily increased, and the patient died suddenly, September 28.

Autopsy.—The postmortem examination was made two hours after death. The body was emaciated. The veins over the anterior thoracic wall were greatly distended. Both arms were edematous; the lower extremities were emaciated but showed no edema. Two brown pigmented areas were present in the skin of the abdomen. The entire left pleural cavity was filled with a solid white mass, which was adherent to the pleura on all sides. The heart was displaced to the right of the midline. The tumor mass could be dissected free by blunt dissection,

with removal of the parietal pleura, except in the region of the third to fifth thoracic vertebrae, where it was firmly adherent to the vertebral column. The mass was roughly oval, with many small knobs of tumor tissue projecting from it. It weighed approximately 3,400 Gm. and measured 30 by 18 by 14 cm. in greatest dimensions. On section it consisted of firm white tissue in which were many yellow-white necrotic areas. In the upper portion was an encapsulated area of tumor, approximately 15 cm. in diameter. In other areas the surface presented a distinctly whorled appearance. The left lung was embedded in the bottom of the tumor mass and was compressed against the vertebral column; its greatest dimensions were 11 by 4.5 by 3 cm. Where the tumor was adherent to the spinal

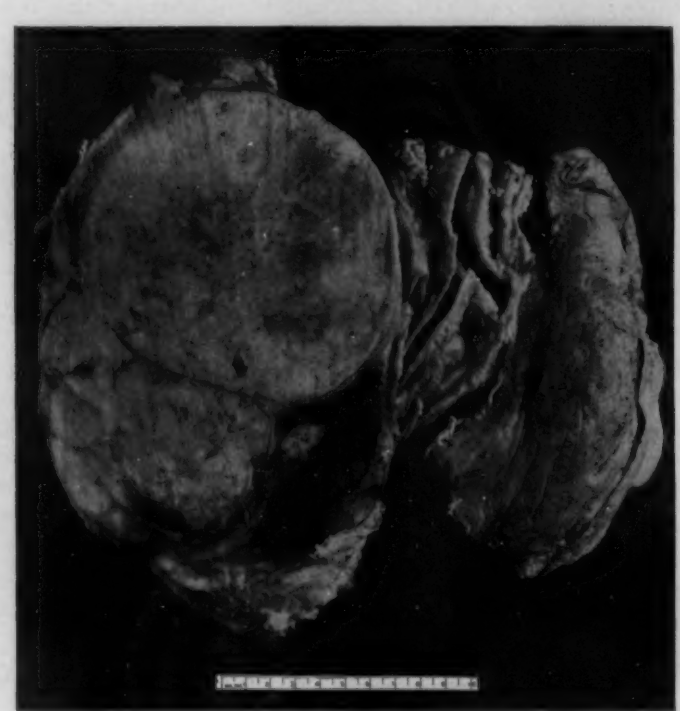

Fig. 2.—The cut surface of the tumor. The compression of the left lung is shown.

column, the vertebral bodies were eroded and infiltrated by the tissue. In the region of the third to fifth throacic vertebrae the spinal column appeared to be deflected to the right by the neoplasm; below the fifth vertebra it made a sharp-angled return to the midline. The second, third and fourth ribs were eroded at the costovertebral junctions.

The right lung, estimated to weigh 200 Gm., was essentially normal, as was its pleura. Multiple sections of the peribronchial lymph nodes revealed no tumor. In the medulla of the right adrenal were two firm white nodules of tumor tissue; the larger measured 2 by 1.75 by 1.5 cm.; the smaller was 4 mm. in diameter. In the upper pole of the left kidney was a white nodule, 4 mm. in diameter, which projected 1.5 mm. above the surface of the surrounding cortex. In the vicinity

of the second lumbar vertebra, just below the origin of the right iliopsoas muscle, was a 3 by 2 by 2 cm. nodule of firm white tissue which projected laterally to the right of the vertebral column and had invaded the bone. The heart, alimentary tract, liver, spleen, thyroid, pancreas, right kidney, left adrenal and generative organs showed only minor gross changes, not related to the main pathologic features.

The histologic appearance of the tumor varied in sections taken from different portions of the large primary growth and from the metastases; the nature of the tumor was best demonstrated in the metastasis in the right adrenal. Here the tumor cells formed interlacing bundles; those cells that were cut longitudinally contained cigar-shaped nuclei, lying roughly parallel and containing a delicate chromatin network. With the phosphotungstic acid–hematoxylin stain the cyto-

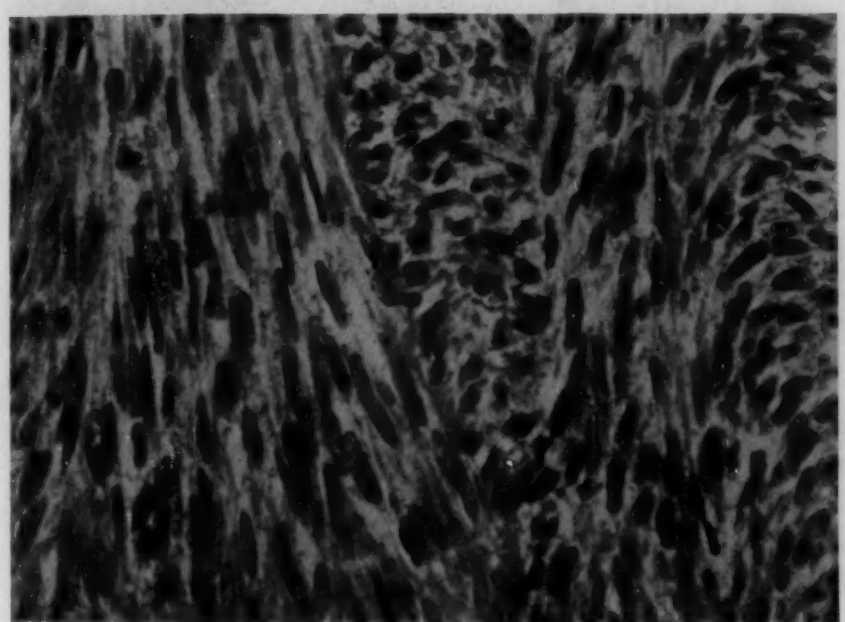

Fig. 3.—Microscopic appearance of the metastasis in the right adrenal; × 520. In these cells myofibrils were demonstrable.

plasm stained pale red, in sharp distinction to the surrounding connective tissue. In many of the cells occasional myofibrils could be demonstrated as delicate blue strands, either extending the length of the cell or appearing to extend from the tip of the nucleus. In others there appeared to have been degeneration of the fibrils with formation of granular material. Adjacent to the longitudinal bundles were groups of cells in which the nuclei appeared in cross section; in a few of these cells cut ends of myofibrils could be seen.

In sections taken from the primary tumor and the metastasis in the left kidney no definite myofibrils were visible. However, in many areas the tumor had the same appearance as in the adrenal metastasis; in other areas the cells were smaller and more widely separated. Mitotic figures were numerous, and there were scattered tumor giant cells. The tumor was well vascularized, but there

were many large and small foci of necrosis, containing and surrounded by polymorphonuclear leukocytes. In sections through the tumor, pleura and lung the tumor tissue had in some places replaced the pleura and was in direct contact with or had invaded the lung tissue. In other regions the connective tissue of the pleura appeared intact and was thickened and vascular, with tumor closely adherent to its external surface. In sections through the tumor and thoracic wall the neoplasm had completely replaced the pleura and invaded muscle bundles. In some places strands of dense connective tissue indicated the probable former site of the pleura. Where the tumor was in contact with the vertebral column, extensive destruction of the bone with invasion of the marrow had occurred; the marrow not replaced by tumor was fibrotic. Similar changes were present in a section of one of the eroded ribs.

In the medulla of the left adrenal was a microscopic tumor metastasis. Multiple sections of the other organs, including the peribronchial lymph nodes, revealed nothing of significance.

COMMENT

It cannot be determined whether this tumor originated in the parietal or in the visceral pleura since it involved both layers and showed extension into the subjacent tissues on both sides. The presence of smooth muscle fibers in the interstitial tissue of the lung and in the pleura was described by Macklin,[5] who found them "in the form of a fenestrated membrane, or in the sheaths about the blood vessels, lymph vessels or bronchi, but yet distinct from the walls of these structures." The existence of smooth muscle in the pleura has also been described by Baltisberger[6] and by Engel and Newns.[7]

SUMMARY

A case of leiomyosarcoma primary in the pleura is presented. Myofibrils were demonstrated in a metastasis. Only a single other instance of leiomyosarcoma of the pleura is recorded in the literature; the case presented here is the first in which metastases were present.

5. Macklin, C.: Physiol. Rev. **9**:1, 1929.
6. Baltisberger, W.: Ztschr. f. Anat. u. Entwcklngsgesch. **61**:249, 1921.
7. Engel, S., and Newns, G.: J. Path. & Bact. **49**:381, 1939.

TERATOMA OF THE SPINAL CORD

MABEL G. MASTEN, M.D., MADISON, WIS.

Teratoma of the spinal cord is rare, according to available reports. Most of those described in the literature have been of bidermal origin. Especial interest is attached to the present report, for it is believed that it concerns a tridermal tumor.

REPORT OF A CASE

A 5 year old girl intermittently complained of pain in her arms three weeks before admission to the Wisconsin General Hospital, April 10, 1939. The pain was referred to the wrists and elbows until a few days before admission to the hospital, when she complained also of pain in her left knee. Stiffness of the neck and

Fig. 1.—Longitudinal section through the teratoma showing in the left half a collapsed cyst lined with a variety of epithelium and to the right of the cyst, glands, ducts, tracheal epithelium and cartilage; × 8.5.

tilting of the head had been noticed by her parents, but muscular weakness had not been noted until the day of admission, when she was observed to use the left hand in eating and to have difficulty in walking. The parents remembered that sometime during the fall months of 1938 she had had "a spell" lasting a few minutes during which she complained of weakness in her legs.

The child was alert but irritable. Her head was tilted to the left and was not moved voluntarily. The neck was stiff, and attempts to flex it resulted in pain. There was generalized hypotonia, more marked in the arms than in the legs. The tendon reflexes were reduced in the arms, there was marked reduction in muscle strength in the right arm, and the right grip was almost powerless although other movements of the muscles of the hand could be performed. The left grip was strong. There were slight weakness and ataxia of the right leg. The reflexes in the right leg were increased, and the abdominal reflexes were absent. She walked

From the Department of Neuropsychiatry, University of Wisconsin Medical School.

Figure 2

(See legend on opposite page)

with a staggering, hemiplegic gait. Cooperation could not be obtained for a sensory examination. Roentgenograms of the spine showed no abnormality.

Lumbar puncture revealed an initial pressure of 13 mm. of mercury, which increased to 20 mm. on compression of the jugular veins. After removal of 10 cc. of spinal fluid, there was abrupt cessation of flow. The fluid contained 1,200 mg. protein per hundred cubic centimeters. Within a few hours after the puncture, the temperature rose to 103 F. and steadily climbed to 108 F. on the third day after the puncture; it remained around 103 or 104 F. to the end; at the same time the pulse became rapid and the respiratory rate increased. Following the puncture, paralysis of the intercostal muscles developed, with a bilateral Babinski sign, absence of knee jerks and dribbling of urine. Early after the spinal puncture the right arm was observed to maintain a position of abduction at the shoulder and flexion at the elbow. Soon thereafter the left arm assumed the same position. If the arms were placed at the sides, they immediately returned to the former position.

It was assumed that there was a tumor of the spinal cord at the level of the sixth cervical vertebra. A laminectomy was done from the sixth cervical to the second thoracic vertebra. Beneath the dura on the posterior aspect of the cord, a small, freely movable, thin-walled cyst was attached to the right side of the cord by a short pedicle. During its removal it ruptured and a grayish sticky fluid was discharged. The patient never regained consciousness and died on the third postoperative day.

Dr. Mead Burke made the following pathologic report:

The immediate cause of death was determined to be acute bilateral bronchopneumonia. The laminectomy wound was uninfected and healing satisfactorily. However, severe pressure damage was obvious in the cord for a distance of 3.8 cm. along and below the cervical enlargement. Grossly this involved, on cross section, an area approximately 2 cm. in diameter, beginning about the lower level of the fourth cervical vertebra and extending throughout the length of the cervical enlargement to the eighth cervical or first thoracic vertebra. Cephalad it was seen only on the right side, involving the posterior and lateral columns. In its course caudad, however, it enlarged and encroached on the anterior column, eventually extending over on the left side to involve the left posterior and lateral columns. Microscopically, the sections all showed large areas of marked degeneration, necrosis and softening, with some recent hemorrhage. Little gliosis was noted. The cystic tumor found at operation had been completely excised.

The tumor was oval shaped, measuring approximately 2.8 cm. in length and 0.8 cm. at its thickest portion; it was partly cystic and partly solid. The wall of the cystic portion felt thin and pliable, and within the cystic cavities, which made up two thirds of the tumor, were several cubic centimeters of translucent gelatinous material of a gray color.

Microscopically, the walls of the cystic cavities showed a relatively simple structure. They were lined by epithelium varying from a single layer of squamous

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EXPLANATION OF FIGURE 2

Fig. 2.—Upper: Anlage of the respiratory tract; × 30. Note mucous and serous glands with ducts directed toward the tracheal structure (center) lined with pseudostratified ciliated columnar epithelium. Hyaline cartilage is seen on the right.

Lower: Ganglions, nerve fibers and nerve cells; × 120.

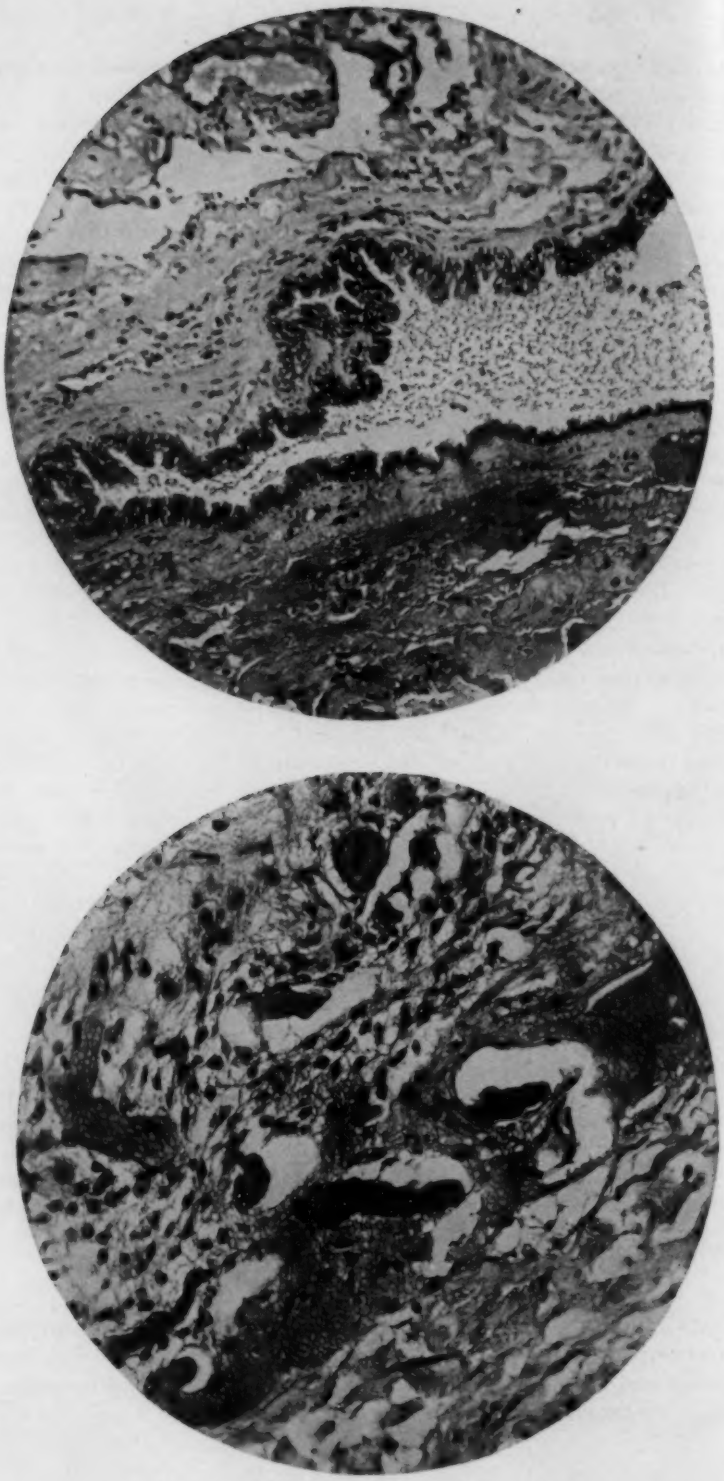

Figure 3

(See legend on opposite page)

cells to a multiple layer of pseudostratified epithelium; certain sections showed a lining of cuboidal epithelium with papillary infoldings. The solid portion, however, presented a much more complex structure. The following tissues and cells were identified: peripheral nerves with numerous ganglions scattered along their courses; nerve cells; a cavity resembling the central canal, lined by ependymal cells and surrounded by glial tissue; pacinian corpuscles; other cells, presumably astrocytes and oligodendrocytes; adipose tissue; collagenous connective tissue; smooth muscle; striated muscle; cartilage; bone; mucous glands; squamous, cuboidal and pseudostratified epithelium, and ciliated columnar epithelium. The nests of mucous glands were numerous and in one area lay between a section of cartilage and a cavity lined by pseudostratified columnar ciliated epithelium. Running through this area were a few bands of smooth muscle, and here and there could be seen thin-walled ducts apparently extending from the glands to the cavity. The individual tissues seen in this area and their definite arrangement in a pattern bear a striking resemblance to the normal tracheal structures, and as such they would arise from the inner germ layer. The mesoderm is represented by voluntary muscle fibers and numerous mesenchymal tissues, including smooth muscle, adipose tissue, cartilage, bone and collagenous connective tissue. A considerable portion of the tumor was made up of nerve tissue of ectodermal derivation.

(Sections were stained with hematoxylin and eosin and by Masson's [1] trichrome method.)

The genesis of teratomatous tumors was thoroughly discussed recently in the reports by Kubie and Fulton,[2] Hosoi,[3] and Bucy and Buchanan.[4] These workers expressed concern over the derivation of the ciliated columnar cells found in their tumors, and in spite of the association, in several instances, of this type of epithelium in logical sequence with mucous and serous glands and cartilage, they concluded that their tumors were of bidermal origin, ascribing the derivation of the ciliated columnar epithelium to the ectoderm. Kubie and Fulton [2] pointed out that within the skin itself the ectoderm rarely gives rise to cysts containing ciliated cells, whereas for the most part such cysts arise only from endodermal vestiges. Voss [5] described a complex teratomatous tumor occurring intradurally at the caudal end of the spinal cord. This was associated with syringomyelic gliosis and cavity formation extending from the seventh thoracic segment to the caudal end of the cord, where the teratomatous cystic tumor was found. Voss discusses the genesis of this tumor, which resembles the subject of the present report, in great detail. He points out that the association of ciliated epithelium, mucous and serous glands, smooth muscle and

1. Masson, P.: J. Tech. Methods **12**:75, 1929.
2. Kubie, L. S., and Fulton, J. F.: Surg., Gynec. & Obst. **47**:297, 1928.
3. Hosoi, K.: Arch. Path. **11**:875, 1931.
4. Bucy, P. C., and Buchanan, D. N.: Surg., Gynec. & Obst. **60**:1137, 1935.
5. Voss, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **163**:289, 1938.

EXPLANATION OF FIGURE 3

Fig. 3.—Upper: Part of cyst wall lined with epithelium varying from low cuboidal to pseudostratified columnar; × 120.

Lower: Striated muscle in center of section; × 120.

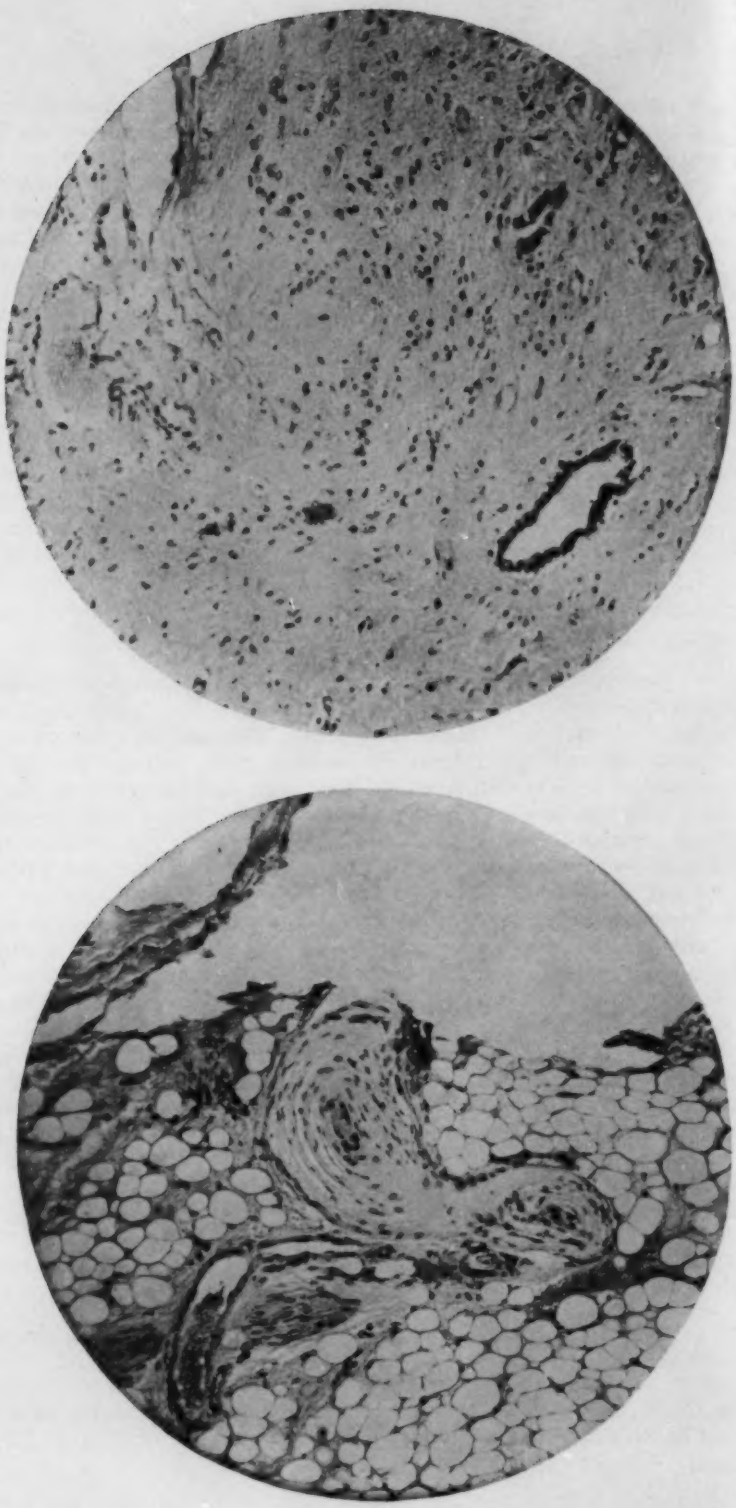

Figure 4

(See legend on opposite page)

cartilage constitutes the anlage of the respiratory tract and is mute evidence of endodermal derivation. On the other hand, Puusepp [6] in considering a less complex teratoma, in spite of its location in the cervical region, interprets the finding of ciliated epithelium, mucous glands, nerve ganglions, lymphoid structures, elastic tissue and muscle fibers as representative of the anlage of the small intestine. In view of the location and the finding of ciliated epithelium it would seem more logical to assume that the organoid structure reported by Puusepp was the anlage of the respiratory tract. Unfortunately, for good interpretation of the Puusepp tumor the histologic description is too brief and the photographic reproductions do not permit close study.

In the case reported here the morphologic similarity to the epithelium of the respiratory tract, with the orderly arrangement of mucous and serous glands, with ducts leading to a cyst lined with ciliated epithelium, and the hyaline cartilage, led to the conclusion that this is an instance of a three germ layer tumor. Hosoi [3] stated that ectodermal and mesodermal elements tend to overgrow at the expense of the endodermal structures, which may either become retarded in their growth or disappear altogether. In the light of this statement it would seem logical to assume that in the highly complex embryonic tumors described in articles gathered from the literature by Hosoi, among which appear several with ciliated columnar epithelium, the endoderm has been represented in a few even though overshadowed by the abundance of ectodermal and mesodermal structures.

SUMMARY

A teratomatous cyst attached to the pia of the lower part of the cervical portion of the cord has been described. It is believed to be the fourteenth teratoma [7] of the spinal cord to be reported, and although it is only the second wherein the author has assumed the presence of third germ layer derivatives, it is probable that there have been other tumors among those reported in which the endodermal structures were represented.

6. Puusepp, L.: Rev. neurol. **2**:879, 1934.

7. The case of A. A. Walker and C. H. Moore (Am. J. Dis. Child. **57**:900, 1939) has been excluded. From the gross and microscopic descriptions of the tumor they have reported, it is obvious that they were dealing with a simple dermoid sinus and dermoid cyst.

EXPLANATION OF FIGURE 4

Fig. 4.—Upper: Glia tissue with cavity lined with ependymal epithelium; × 90. Lower: Pacinian corpuscles surrounded by fat; × 120. Note blood vessel in the left lower field.

MALIGNANT MELANOMA OF THE PALATE

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Territory of Hawaii

Melanoma most commonly arises from the choroid coat of the eye or from a pigmented mole of the skin. Less frequently it is primary in the arachnoid, anus, rectum and other structures.[1] In rare cases no focal point can be demonstrated.[2] Melanoma having its origin in the hard palate has been reported.[3] Because this site is infrequent, we feel that a case we have observed is worth presenting.

REPORT OF A CASE

A feebleminded Japanese woman, aged 25, was admitted to the Queen's Hospital, May 5, 1939, with the complaints of swelling of the roof of the mouth and generalized weakness.

Her past medical history was irrelevant except for the fact that dark pigmented spots had been present on the hard palate since very early childhood. This pigmentation became more obvious in the latter part of 1938. During the next six months these bluish areas became slightly elevated and confluent and turned coal black. As this process spread, the upper right and anterior gingival margins became involved, causing the teeth to become loosened and to turn dark. Extraction of these loose teeth caused an apparent exacerbation of the growth. In April 1939 the hard palate, including the gums, had become a black spongy mass, which bled on the slightest trauma.

The patient was a slightly built Japanese woman, not acutely ill. Her eyes and eyegrounds showed no gross changes. Her nose and nasopharynx were normal. Her mouth presented a very striking picture (fig. 1). In the area of the hard palate and gums of the upper jaw was a painless, spongy, friable, black, irregular mass, which oozed blood when irritated. The only upper teeth present were the left lateral incisor and the premolars. The remainder were absent, and the gums were completely replaced by tumor tissue. Many of the lower teeth were missing. Those present were in good condition and firmly embedded. The anterior cervical lymph nodes on the right side were discrete and palpable. The chest showed no definite percussion changes. The breath sounds were normal, but there were many fine rales, heard best at both pulmonary bases,

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From the Queen's Hospital Tumor Clinic.

1. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928. Akelaitis, A. J.: Am. J. Path. **11**:591, 1935. Jaleski, T. C., and Waldo, P. V.: Am. J. Cancer **24**:340, 1935.

2. Plewes, F. B.: Am. J. Cancer **26**:732, 1936.

3. Bernstein, J.: J. Laryng. & Otol. **44**:328, 1929. New, G. B., and Hansel, F. K.: J. A. M. A. **77**:19, 1921. Takezawa, N.: Oto-rhino-laryng. **11**:234, 1938. Patterson, N.: J. Laryng. & Otol. **41**:32, 1926.

posteriorly. The heart was normal in size and shape. No murmurs were heard. The blood pressure was 120 systolic and 80 diastolic. The abdomen was soft, and no masses or hernias were present. The vagina and rectum were normal. Neurologic examination showed no positive abnormalities.

Roentgenograms of the chest showed fine miliary metastases in both lower pulmonary fields. A roentgenogram of the skull showed some clouding of the right antrum with slight absorption of the malar bone on the same side. The hemoglobin content was 70 per cent; the red cell count was 3,800,000, and the white cell count was 7,000, with 70 per cent polymorphonuclear leukocytes, 23 per cent lymphocytes, 1 per cent monocytes and 1 per cent eosinophils. The urine had a specific gravity of 1.010, gave an acid reaction and showed no albumin and no sugar. The microscopic examination showed an occasional leukocyte with some stratified squamous epithelial cells. No melanin was detected in the urine. The Wassermann and Kahn tests were negative.

A biopsy of the tumor from the region of the hard palate established the diagnosis of malignant melanoma.

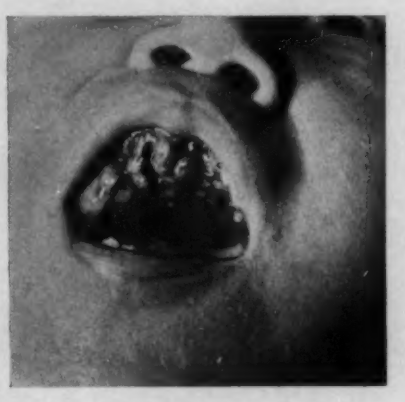


Fig. 1.—Appearance of the patient showing tumor of the hard palate.

The general condition improved considerably with rest in bed and a high caloric, high vitamin diet. On May 13, 1939, treatment with roentgen rays of high voltage was begun over the tumor mass involving the region of the hard palate. The total dose was 1,400 roentgens, with 0.5 mm. of copper and 1 mm. of aluminum as filters, at a distance of 50 cm. This was administered in divided doses, a treatment being given every day over a period of three weeks. The patient was discharged from the Queen's Hospital May 15. She returned as an outpatient until the course of roentgen treatment was completed.

The tumor did not respond to these treatments, and consequently the treatments were discontinued. There was no opportunity to observe the patient from this time until her death, November 20.

Autopsy.—The body was that of a young, poorly developed and markedly emaciated Japanese female. There was no jaundice. The pupils were round and equal, and the sclera was clear. The nasal passages were normal. The mouth showed a protruding mass of black, soft, friable tissue covering the entire hard palate and extending to involve the gums. The only upper teeth remaining were the left lateral incisors and the premolars. These had become surrounded and loosened by the tumor invading the gums. On palpation the growth was a soft

nodular mass as felt against the bony palate. The tongue was not involved. The remaining oral membranes were intact. There were a number of soft palpable glands in the submaxillary areas. The chest was of average proportions. The sixth rib on the right side in the anterior axillary line showed a nodule 3 cm. in diameter. The skin was normal. There was no dependent edema.

Metastatic tumor tissue was found throughout the lungs, in the ribs, skull, meninges, pleura, epicardium, endocardium, peritoneal surface, liver, gallbladder, pancreas, kidney, gastrointestinal mucosa and bladder mucosa and in many areas of the brain. There was a metastasis in the midpons, 1 cm. in diameter, occupying almost the entire right half of the pons in the region of the pyramidal tract.

Microscopically, the tumor tissue consisted of compact groups of cells with a definite epithelial character. The predominant type was a moderately large cell

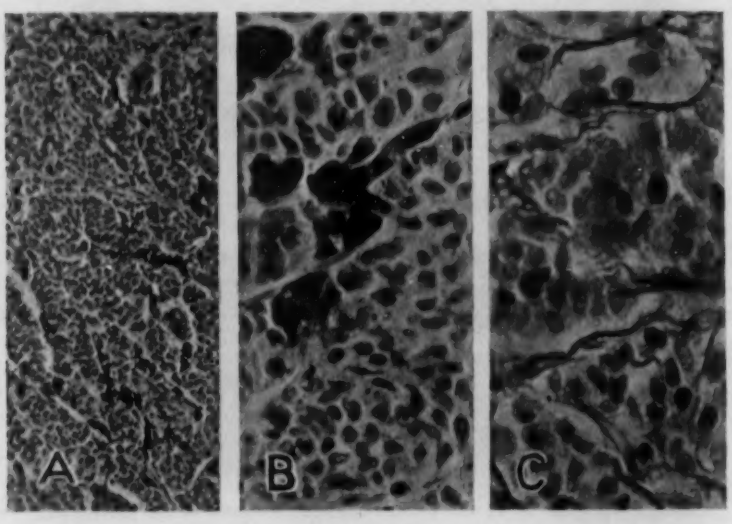


Fig. 2.—*A*, low power view of tumor taken for biopsy; *B*, high power view of tumor metastasis; *C*, reticulum stain of tumor.

with distinct cytoplasm, frequently containing brown granular pigment. The nuclei were large, rounded or oval, hyperchromatic and rather uniform in size, with prominent nucleoli. The stroma was relatively dense and variable in amount; it contained large masses of granular brown pigment. Metastases were identified in the lungs, heart, brain, liver, pancreas, kidneys, adrenals, bladder and uterus. The quantity of pigment within tumor cells varied. Isolated tumor cells were noted in the blood vessels. The vascular system showed leukocytes containing a large number of pigment granules, and similar pigment granules were noted free in the lumen. Other microscopic sections disclosed a similar evidence of an excess of pigment in the endothelium of many vessels, Kupffer cells of the liver, endocardium, renal glomeruli and tubular epithelium.

A reticulum stain (silver carbonate) indicated the presence of a relatively small number of argyrophil fibers, seen only in the heavier stroma and about the periphery of the cell masses (fig. 2*C*).

COMMENT

The case presented is unusual because of the origin of the tumor and its extensive spread. The palatal pigment had been present since early childhood, but the tumor was first observed in January 1939. The diagnosis was established by biopsy on May 5, 1939. This time a roentgenogram of the chest showed miliary metastases in the lungs. This somewhat indefinite onset makes any estimation of the rate of growth and of the malignancy unsatisfactory. Histologically, the tumor tissue appeared relatively active, and this impression was apparently confirmed by the scarcity of argyrophil fibers (fig. 2 *C*), a method used by Callender and Wilder [4] in estimating the relative malignancy of melanomas of the choroid.

The patient was declared feebleminded in 1935 and was admitted to a mental institution at the age of 21. In this connection, no relationship between the pigmented palate and the stigmas of degeneracy could be determined. Unfortunately, there is no information as to the type and structure of the hard palate before the extensive growth of the tumor, and this could not be determined at autopsy.

SUMMARY

A case of widespread malignant melanoma originating in the region of the hard palate is presented.

4. Callender, G. R., and Wilder, H. E.: Am. J. Cancer **25**:251. 1935.

Laboratory Methods and Technical Notes

A RAPID METHOD OF STAINING FAT IN FROZEN SECTIONS WITH OSMIC ACID

ARAM A. KRAJIAN, LOS ANGELES

For osmic acid staining of fat in tissue, most of the works on histologic technic describe an elaborate and rather unsatisfactory method of whole block staining and sectioning by a modified paraffin technic. Rapid dehydration and cedarwood oil clearing, which are necessary to prevent loss of fat, add to the difficulty of the method.

Mallory recommends a frozen section method as more satisfactory, but the technic described requires about forty hours for the preparation of a section.

As a differential fat stain, osmic acid occupies an important position. According to the classification of fats worked out by Black,[1] mineral oils are stained orange red by scarlet red and are not stained by osmic acid.

In the investigation of the character of the fat causing lipoid pneumonia, osmic acid is an important aid, and a method for rapid diagnosis would be particularly helpful.

Here I describe a rapid method of staining fat with osmic acid, only ten minutes being required to cut, stain and mount the section.

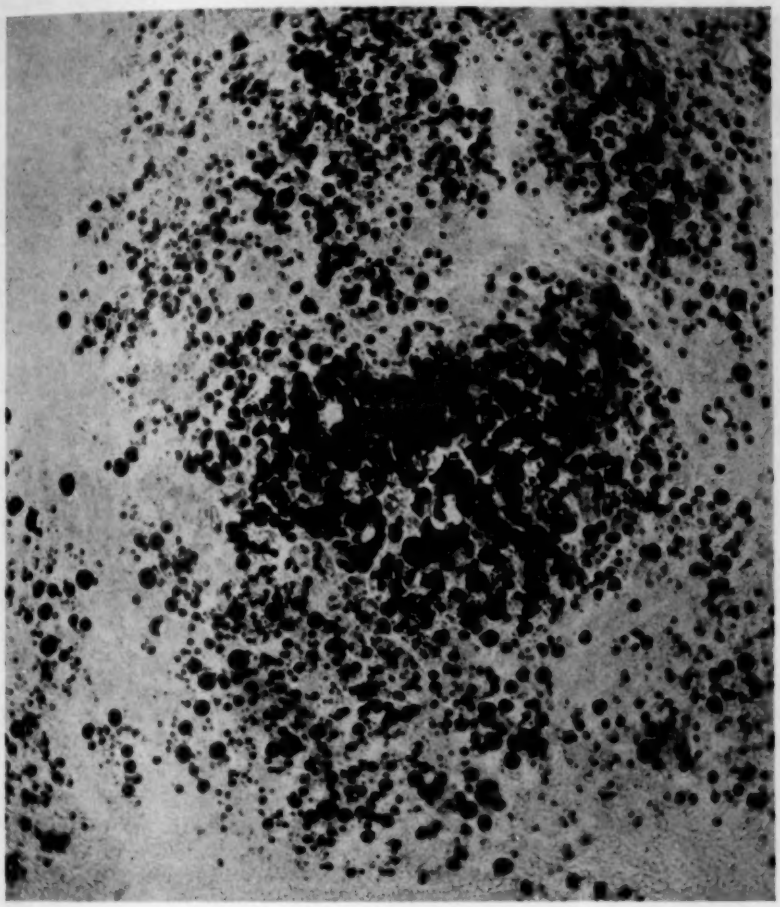
METHOD

1. Fix tissue in a 10 per cent solution of formaldehyde for twenty-four hours.
2. Cut frozen sections 10 microns thick.
3. Bring to a boil a 1 per cent aqueous solution of osmic acid in a pyrex test tube and pour the solution into a small Stender dish.
4. Place the section in the hot osmic acid solution and keep in a paraffin oven at 60 C. for five minutes.
5. Wash in a basin of tap water.
6. Counterstain in 1 per cent aqueous eosin or phloxin for one minute.
7. Wash rapidly in tap water.
8. Transfer the section to a slide and let drain off.
9. Place 3 large drops of glycerin jelly (previously melted in a paraffin oven or a water bath) and cover with a cover slip.

1. Black, C.: J. Lab. & Clin. Med. **23**:1027, 1938.

Result: The fat globules are black or gray-black; the background is red.

For emergency examination, bring a 10 per cent solution of formaldehyde to boil in a pyrex test tube (60 cc. capacity); drop a thin piece of biopsy or autopsy material into it and place the specimen in a hot oven (60 to 65 C.) for ten minutes; then cut thin frozen sections and stain by the method described.



Liver cells showing fatty infiltration stained with osmic acid by the method described; × 100.

It is not necessary to discard the used osmic acid solution. Pour it back into the stock bottle and use it over and over.

After the stain has been used about a half-dozen times, control sections of known osmic acid–staining fat should be made each subsequent time to check the efficiency of the stain.

A METHOD FOR STAINING MICROGLIA

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Since Río Hortega's description [1] of microglia and of his first method of impregnation of the microglia by silver carbonate, various other technics have been advanced purporting to improve the demonstration of these cells, especially in material fixed in dilute solution of formaldehyde, or in old material, or to hasten staining.

Río Hortega [2] modified his first technic, of 1920, in 1927 by using a strong silver carbonate solution (5 cc. of a 10 per cent solution of silver nitrate, 20 cc. of a 5 per cent solution of sodium carbonate and ammonia in sufficient quantity to dissolve the formed precipitate), thus obtaining better results in the impregnation than had been possible with his original weak silver carbonate solution. In addition, he was able to use material which had been very briefly fixed in solution of formaldehyde.

Río Hortega's [3] method of ferric impregnation gives different images and may be used in connection with some degenerative processes. A prussian blue color of the finest ramifications containing iron and a rose color of the nuclei may be obtained.

Not as clear or as constant in demonstrating microglia are Cajal's [4] two variants of his pyridine–ammoniacal silver technic, originated for fibrous neuroglia. One of these methods requires from twelve to twenty-four hours for impregnation; the other, one month in a 15 per cent dilution of solution of formaldehyde U. S. P. after twelve to twenty-four hours of fixation.

Gans [5] studied the microglia in old material, fixed in a dilute solution of formaldehyde and strongly bromated with hydrobromic acid before impregnation with the weak silver carbonate solution of Río Hortega's 1921 formula (10 cc. of a 10 per cent solution of silver nitrate, 30 cc. of a 5 per cent solution of sodium carbonate, ammonia sufficient to dissolve the precipitate and distilled water to make 150 cc.); this required a minimum of four to five hours for the bromation and two more for the impregnation.

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* Dazian Fellow in Pathology.

1. del Río Hortega, P.: Trab. d. Lab. de invest. biol. Univ. de Madrid **18**:37, 1920.

2. del Río Hortega, P.: Mem. r. Soc. españ. de hist. nat. **27**:199, 1927.

3. del Río Hortega, P.: Bol. r. Soc. españ. de hist. nat. **27**:372, 1927.

4. Ramón y Cajal, S.: Trab. d. Lab. de invest. biol. Univ. de Madrid **23**:157, 1925.

5. Gans, A.: Ztschr. f. wissensch. Mikr. **40**:311, 1923.

Bolsi[6] described another technic, utilizing a 2 per cent solution of silver nitrate subsequent to fixation of fresh material in a fluid made of pyridine, acetone, solution of formaldehyde, distilled water and ammonium bromide. After a period of fixation lasting from six days to two months, eighteen to twenty-four hours of washing are required.

Penfield[7] developed a method in which he used Globus'[8] hydrobromic acid and a mordant solution of sodium carbonate before impregnation with a weak silver carbonate solution (Río Hortega's 1927 strong silver carbonate solution by adding 75 cc. of distilled water). This method is fairly long.

A new technic has been developed for the study of microglia, based on some observations made while studying (with Dr. J. Bofill and Dr. A. R. Zamora) the action of potassium cyanide, among other compounds, on diverse elements of several tissues.[9] It was subsequently used with the Río Hortega weak silver carbonate solution.

This technic is a distinct improvement over those most commonly used today and noted in the foregoing paragraphs. It has a wider field of usage and is a great time saver.

METHOD

1. Harden: in a 10 per cent dilution of solution of formaldehyde U. S. P. or in solution of formaldehyde U. S. P.–ammonium bromide for an indefinite period.

2. Section: frozen sections from 15 to 25 microns thick.

3. Wash: (1) in a solution of equal parts of alcohol, ammonia and pyridine for ten minutes; (2) thoroughly in distilled water for one minute.

4. Cyanurate: thirty minutes in a dish used for silver staining, containing a 10 per cent solution of potassium cyanide (KCN) at a temperature of 45 to 50 C. (This temperature can be obtained by heating the solution over an alcohol lamp. This should be done under a hood or in a ventilated room, to avoid the dangers of cyanide fumes.)

5. Wash: thoroughly in distilled water three times.

6. Impregnate: with Río Hortega's weak silver carbonate solution at room temperature. After the first five seconds, keep a second to second check on the sections, to control the impregnation time. (Río Hortega's weak silver carbonate solution: silver nitrate, 10 per cent, 5 cc.; sodium carbonate, 5 per cent, 20 cc.; ammonium hydroxide, sufficient to dissolve precipitate; distilled water, 75 cc.)

7. Reduce: in 1 per cent dilution of solution of formaldehyde U. S. P. Agitate the section by blowing on the surface of the formaldehyde solution after the sections have been immersed. Both the impregnation and the reduction are carried out one section at a time.

8. Wash: lightly in distilled water.

9. Tone: in 1:500 gold chloride solution at laboratory temperature until yellow spots disappear and a uniform gray is obtained. (This step may be improved by slight heating over an alcohol lamp.)

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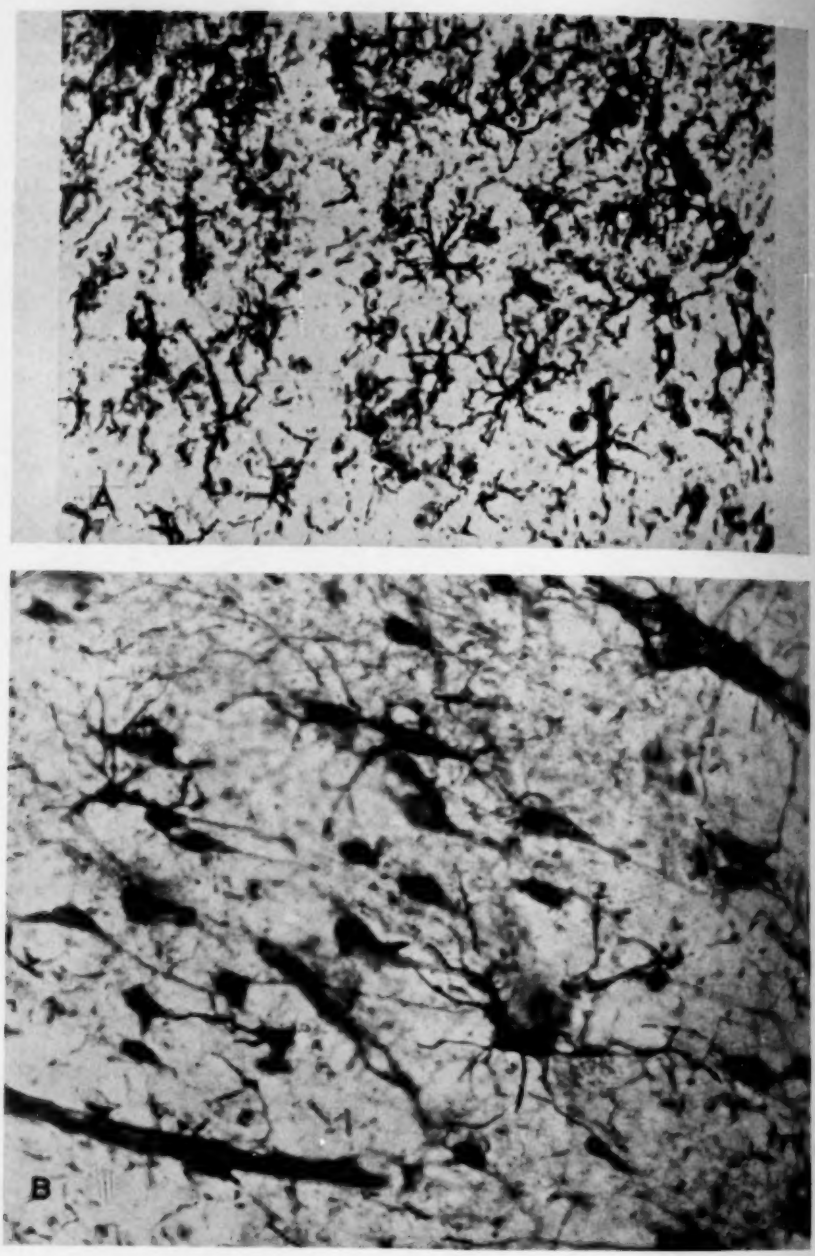
6. Bolsi, D.: Riv. di pat. nerv. **32**:898, 1928.

7. Penfield, W.: Am. J. Path. **4**:153, 1928.

8. Globus, J. H.: Arch. Neurol. & Psychiat. **18**:263, 1927.

9. The findings were not published at the time, owing to special circumstances having no relation to the work itself.



A, microglia in a case of infection with Torula histolytica, fixed six months in diluted solution of formaldehyde U. S. P. (1 : 10). *B*, the same tissue under higher power magnification.

10. Fix: in a 5 per cent solution of sodium hyposulfite until the flexibility of the sections, lost in the gold chloride solution, is restored.

11. Wash; dehydrate with absolute alcohol; clear with a few drops of carbol-xylene-creosote, and mount in Canada balsam.

The solutions are made with distilled water and the sections are washed with distilled water. The general precautions for any silver impregnation should be kept in mind.

This method gives good results (figure) and offers the following advantages: 1. No special fixation is required (either solution of formaldehyde U. S. P. or solution of formaldehyde–ammonium bromide may be used). 2. Sections of old material may be impregnated. 3. It is constant, offering clear and good images, especially of pathologic forms. 4. It permits economy in the use of sections inasmuch as even those not showing perfect impregnation may be restained; it is only necessary to repeat the steps of this technic, beginning with the stage of cyanuration. In no way does it impair the previous impregnation. 5. It requires only about one hour.

General Reviews

HISTOLOGY OF TUMORS OF THE PERIPHERAL NERVES

NATHAN CHANDLER FOOT, M.D.
NEW YORK

In this article an attempt will be made to evaluate present histologic knowledge of tumors of the peripheral nerves, some of which are well understood while others are still the subject of lively controversy. At the time Rudolf Virchow devoted 72 pages of his book "Die Krankhaften Geschwülste" to "neuroma" the subject was an open field in which the author had a virtual monopoly of ideas. Knowledge of "neuroma" was acquired through gross examination and by means of a very simple microscopic technic that utilized primitive staining methods or, dispensing with these, depended on the observation of unstained teased material studied in a macerating fluid such as dilute sodium hydroxide.

Since 1863 histologic knowledge of the nervous system has increased enormously as the result of a corresponding multiplication of the avenues of approach. The technic of staining tissues has been improved to a point that seemingly approaches completeness, silver and other metallic impregnations have been devised, and much that was hitherto invisible has been brought to light through their medium, although, unfortunately, there are still far too few pathologists who are ready to avail themselves of this extensive armamentarium.

As normal histologic appearances have become better understood, investigators in allied fields have been called into cooperation, and those in the fields of comparative histology and embryology have added much to that which was formerly available. Workers in the field of experimental biology and embryology have also played their part in contributing facts that were otherwise unsuspected and, as a result, the subdivisions of those branches of investigation that deal with pathologic aspects have likewise acquired much wider scope.

Along with this accretion of factual data, however, has come a corresponding amount of confusion; things that once seemed simple have become complex, and one is faced with controversies that seem impossible of solution through the means at hand, questions that never troubled the older pathologists, because they never suspected their existence.

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For the purposes of this review it will be necessary to consider and discuss the various forms of tumors of the peripheral nerves in turn, briefly mentioning those that are well understood and giving more extended attention to those that remain a bone of contention in spite of all the advances in this field. In order to do this it is proposed to follow a classification that will indicate the histogenetic position of each tumor in a scale. First, however, it will be well to list a number of general articles to which the reader may turn for orientation.

The tumors of the peripheral nerves are discussed at length in the chapters of Penfield's system, the "Cytology and Cellular Pathology of the Nervous System," each written by an outstanding authority, to whom the editor has granted much latitude of thought, so that there is enough stimulating contradiction in these volumes to provoke the curiosity of the reader. Stout, Geschickter, Lewis and Hart, Schultz and Foot have published general articles within the last decade or so, Adair and McLean have analyzed data on a series of peripheral nerve tumors, and Cutler and Gross have contributed to the subject through their paper on the surgical consideration of similar neoplasms. Roussy, Lhermitte and Cornil have written an excellent paper, in which they discuss their classification at length and append a long list of references. Their article is of value to those who wish to obtain an idea of the French point of view. The paper of Fittipaldi discusses the same subject and serves the same purpose for the Italian literature. As far as the German school is concerned, almost any paper of German origin listed at the end of this section will be found to cover the subject exhaustively.

One finds, on reading these papers, that the conceptions as to what constitutes tumors of the peripheral nerves differ somewhat. In Virchow's day any tumor of the nervous system was some sort of "neuroma," provided that it was in part composed of neurofibrils. Nowadays one is confronted with the fact that the nucleus of some future neoplasm may be cast up at some point quite distant from the central nervous system, where it originated, by abnormal embryonal migration. Such a tumor is histologically more closely allied to that system than to the peripheral nerves. Neuroglia, formerly supposed to reside exclusively in the central nervous system, now crops out in tumors of the sympathetic chains and the peripheral nerves, and the term "peripheral glioma" is sometimes used in describing such dislocated tissue. Tumors apparently predominantly of nerve sheath origin may contain elements that closely resemble embryonal neuroepithelium. Neuroepithelial tumors have been noted in serous membranes. Nerve terminals, or "end organs," apparently form tumors formerly attributed to proliferation of epidermal elements, so that those growths, too, must be included in the category of tumors of peripheral nerves. Such neurovascular organs as the cutaneous glomuses give origin to neoplasms,

largely vascular to be sure, but definitely in line with the peripheral nervous system. The question is further complicated by the paraganglionic growths. Should these be included in this category? The fact that they may have a mixed composition that combines gangliar and paragangliar elements rather indicates that they should.

One more question as to the scope of the title of this review is occasioned by the protean manifestations of von Recklinghausen's neurofibromatosis, in which many tumors occur with more or less regularity and yet have no apparent relationship to nerve tissue as such. Multiple forms of lipoma, myxoma and possibly pure fibroma and the multiple foci of sebaceous gland hypertrophy seen in Pringle's disease, followed by multiple keloid fibroma, form a confusing group that is usually included in the concept neurofibromatosis. Perhaps the best way to consider these is to include them among tumors of the peripheral nerves in the sense that they are associated with these nerves even though they do not arise directly from them.

The histology of the nerve trunk seems quite simple and well understood if one reads but a single authoritative paper on the subject, but should one read several it will take on more and more complexity. Before one may intelligently consider tumors of nerves one must recognize this element of uncertainty and realize that it forms a certain barrier to a complete understanding of these growths. If one is uncertain concerning the normal, what can one understand of the variations that lead to the abnormal? For this reason it is proposed that the fundamental histologic components of the peripheral nerve trunks be described in some detail, for this may be of value when the tumors come under discussion.

COMPONENTS OF NORMAL PERIPHERAL NERVE TRUNKS

It is well known that the functional portion of a peripheral nerve is the neuraxon and that this, running out from a more or less centrally located nerve cell, is associated, in a bundle, with like axons that are bound together by various sheaths to make up the nerve. Around each neuraxon is a sheath, and it is from this that the peripheral neurogenous tumor develops in most instances, not from the neuraxon. The myelinated nerves have their myelin sheaths and the syncytial sheath of Schwann; the nonmyelinated nerves lie directly within cellular syncytia that are composed of cells resembling those of Schwann, form retiform complexes and are known as Remak's fibers.

The sheath of Schwann has a limiting membrane, the membrane of Schwann, outside of which is a connective tissue sheath, the endoneurium. This is continuous with perineurium that binds a bundle of such structures together to form a fascicle. The endoneurium contains longitudinal fibrous thickenings that run parallel with the long axis

of the neuron, the fibers of Key and Retzius, and between these is a membrane known as that of Plenck. In this Laidlaw was able to impregnate a subsidiary meshwork of fibers and to show that the endoneurium may start at a ganglion cell, which it surrounds, and continue out along the course of its neuron. Where nerves enter the cord, however, the fibers of Key and Retzius spread out into the external layers of the intima piae, while the finer Plenck-Laidlaw network continues on for a considerable distance into the white matter before terminating in a nipple-like constriction.

The epineurium is a membrane enclosing the entire nerve trunk and is composed of mixed collagenous and reticulin fibers.

While some nerve trunks are comparatively simple, being composed of myelinated fibers having a motor or a sensory function, others are more complex and contain not only fibers of mixed functions but sympathetic fibers as well. These run along with the myelinated bundles in groups of parallel fibrillae that are readily confused with the fibers of Key and Retzius. The interpolation of occasional ganglion cells in some nerve trunks further adds to the composite character of such a potential tumor primordium.

As one approaches the end of the nerve, the sheath dwindles as the nerve grows smaller and finally the endoneurium and the perineurium fuse into a thin endothelioid pellicle, the membrane of His. The terminals of the nerves are sufficiently complicated and varied to merit a separate chapter, by Boeke, in Penfield's system. The motor neurons end in a variety of forms of "end plate" or as small budlike terminations. The sensory fibers usually exhibit corpuscular organs that vary a great deal in their structure. Thus one has the pacinian corpuscles, the Wagner-Meissner tactile corpuscles, the Golgi-Mazzoni and the Ruffini subcutaneous corpuscles and the end bulbs of Krause, the last lying in the conjunctiva. Similar structures are scattered about the body, too minute to attract attention unless special staining methods and acuity are used for their detection.

Although pathologists have a large number of methods for demonstrating nerve elements, these methods do not always show enough specificity; this is particularly true in regard to the Schwann cells, for which specific methods have been claimed, though they give disappointing results when one desires to rely on them. Bailey and Herrmann stressed this in an article that will be drawn on later. The Schwann cell is supposed to stain red in Masson preparations; Schwann's sheath is supposed to resist dilute acetic acid, which swells collagen. Tarlov attempted to solve the problem by employing the Dockrill modification of the del Rio Hortega silver technic, but it will be seen later that his results have not been too satisfactory.

As a consequence of this, much work on the origin of the Schwann cell has been of an experimental nature. Murray, Stout and Bradley,

following Ingebrigtsen's lead, have grown them out in tissue cultures. Stone has demonstrated the multipotentiality of the neural crest cell, which can produce tissue hitherto ascribed to the mesoderm. Harrison's historic work, the experimental destruction of the neural crest in the frog embryo, with resulting failure of development of sheaths in the posterior nerve roots, is well known. Harvey and Burr, and these with Van Campenhout, as well as Raven, have done similar experiments with the closely related meningocytes. Van Campenhout has confirmed Harrison's observations and shown that the removal of the entire neural crest from the embryo of Rana pipiens will result in total failure of development of the spinal and sympathetic ganglions throughout the trunk region. The motor neurons develop normally but are sheathless; the chromaffin apparatus of the adrenal gland does not develop, and there are no sympathetic nerve elements in the wall of the digestive tube. Such embryos will live as many as forty-five days after the operation.

Nageotte summed up the situation as follows: "This conception of a common origin of neurones and sheaths which, in my opinion, has been demonstrated by Harrison for the spinal roots, seems capable of generalizations for the entire peripheral nervous system. . . . It is a problem difficult of solution by the methods of normal histology; it can be settled only by experiment."

Bailey and Herrmann expressed considerable irritation over the unsolved problems in the histology of the nerves. They recognized three chief groups of structures in the nerve trunk: (*a*) nerve cells, axis-cylinders (neurons) and myelin sheaths; (*b*) capsule cells of ganglions, Schwann and Remak cells, and (*c*) endoneurial and perineurial connective tissue. They were uncertain as to whether the Plenck-Laidlaw sheath lies within or outside the membrane of Schwann. They did not accept the view that the endoneurium and perineurium are of neural crest origin and rejected the presence of intracellular "fibroglia fibrils" in the sheath cells (advanced as proof of mesenchymal origin by Mallory and Penfield) as unimportant, since other cells show very similar fibrils in material that has been fixed in Zenker's solution.

The histology of the nerve sheath has been discussed at somewhat tedious length in the foregoing paragraphs in order to orient the reader and to convince him that a carrying-over of these questionable points into the domain of histopathology will complicate matters to a still more confusing degree. The finer details of the structure of the myelin sheath and the like have been omitted as of little importance in connection with the histogenesis of tumors.

Most of the elements of the nerve trunk listed here are capable of forming some kind of tumor, and most of these growths are of a composite nature, as the interrelationship of the various components of the

nerve trunk is extremely intimate. Experiments by Ingebrigtsen, Nageotte and Spielmeyer show that the Schwann cell, or lemmocyte, seems to have the greatest proliferative drive in nerve repair after injury, the connective tissue elements playing the part of "basting" to hold the severed trunks together until the lemmocytes can build a pathway through the scar for the neurons as they, in turn, regenerate. Should they fail to do so, the lemmocytes will form what Nageotte called "travesties of nerves," nerves complete in all particulars save for the essential central axons. Should a tumor, say neurofibroma, do exactly this, one can readily see how the resulting growth might be interpreted as constituting neuroma.

CLASSIFICATION OF TUMORS OF THE PERIPHERAL NERVES

Before considering the various forms of neurogenous tumors, one must have some sort of classification on which to base the discussion. Most of the older schemes are founded on clinical data and gross appearances, with the microscopic features and the histogenesis of the tumors playing a subsidiary part. One of the most complete is that of Stout, which appears in the 1940 edition of his textbook on human cancer, but it is largely based on clinical characteristics. For the purposes of this review there should be a classification that stresses histogenesis and one that does not separate the malignant from the nonmalignant tumors but brackets them together so as to show their relationship to each other.

A tumor is generally accepted as being a local overgrowth of cells exhibiting autonomous growth and serving no useful purpose; many "tumors" of the nerves are the product of the overgrowth of cell processes, the parent cell being far removed from the site of the "tumor" and showing no visible change. For this reason true neuroma should be composed of neurocytes or their embryonal precursors, as the inclusion of their proliferating processes in the category of tumors is something quite unique in tumor classification. No other tumor group is composed of cell processes. Nevertheless it will be necessary to include them for the sake of convenience and because it has been done for so long, but with the clear understanding that this is done quite consciously.

The inclusion of a large group of neoplasms of the autonomic nervous system in the classification may seem strange, but many of these are peripheral and have been described as inclusions in nerves like the median and the ulnar.

In the scheme of classifications that follows, synonyms are given in parenthesis.

Neuroma

1. Cellular:

(*a*) Primitive and undifferentiated:

Sympathogonioma (neuroepithelioma, neurocytoma)

(*b*) Incompletely differentiated:
Sympathoblastoma (sympathicoblastoma, neuroblastoma)
Medulloblastic { sympathetic neuroblastoma / sympathetic spongioblastoma }
Pheochromoblastic pheochromoblastoma

(*c*) Well differentiated:
Ganglioneuroma
Pheochromocytoma (chromaffinoma, paraganglioma, argentaffinoma)

2. Fibrillary:
(*a*) True: Fibrillary neuroma
(*b*) False: Traumatic neuroma
Appendical neuroma

Tumors of Nerve Sheaths

1. Simple or "specific":
(*a*) Poorly or incompletely differentiated:
Neurilemosarcoma (malignant schwannoma)
(*b*) Well differentiated:
Neurilemoma (perineural fibroblastoma, neurinoma, schwannoma, peripheral glioma)
A type: With whorls or Verocay bodies
B type: Myxomatous with fusicellular components

2. Compound:
(*a*) Poorly or incompletely differentiated:
Neurogenous sarcoma
(*b*) Well differentiated:
Subterminal (cutaneous neuroma)
Sheath neurofibroma
Plexiform neurofibroma
Neurofibroma (*Rankenneurom*, elephantiasis neuromatodes)
Neurofibromatosis (von Recklinghausen's disease)
Associated tumors of neurofibromatosis:
"Sebaceous adenoma" (Pringle's disease)
Lipoma
Fibroma

Terminal Neurogenous Tumors

1. Simple:
(*a*) Poorly differentiated:
Malignant melanoma (melanosarcoma, melanocarcinoma)
(*b*) Well differentiated:
Melanoma
Neuroxanthoma (?)

2. Compound or associated:
(*a*) Glomic tumors
(*b*) Dermatomyoma

Like all classifications, this is merely an approximation, and no hard and fast lines can be drawn between the various types. Combinations

are much more prone to occur in this group of neurogenous tumors than in those outside the nervous system, as they are fundamentally compound tumors in many instances. In discussing them I shall adhere to the subject matter of the classification without necessarily following its order.

NEUROMA

Virchow described the neuroma in 1863 from the standpoint of gross pathology and by means of the simple technic already mentioned. He believed that pure neuroma was a rarity and that "in every instance the neuroma is a composite, organoid tumor." All tumors of nerve sheaths, then, were "neuromas" that contained admixtures of various tissues. Should the growth present giant, or multinucleated, cells, it was probably ganglioneuroma. He described pure nerve trunk tumors that might stand up reasonably well under some modern standards, but he included among these, tumors of the acoustic nerve and tumors of the central nervous system that would now fall under headings outside the neuroma class. In closing he said: "The true neuroma is, according to our experience up to the present time, an essentially local and benign tumor. The extremely rare cases of heterotopic development fall so definitely into the realm of teratology that they may be left out of consideration of our main thesis."

During the past eighty years the large group of tumors which Virchow held to be neuroma has been split up into a number of classes that aline many of its former members among tissues that are not truly neurogenous. With time, the sharp distinctions between central and peripheral tumors have tended to be wiped out, but Virchow's division of these tumors into cellular and fibrillary types is still useful.

SYMPATHOGONIOMA

Tumors of this group, also known as neuroepithelioma, may be found in the central as well as in the peripheral nervous system. They are composed of very primitive cells that retain the characteristics of embryonal neuroepithelium and tend to form distinctive rosettes of cells about a central lumen. Other cells may be fusiform or polar, which has led to some confusion in the past. Tumors of this sort may occur in connection with peripheral nerves; Stout reported 1 in the ulnar, Penfield, Borchardt and Cohn 1 each in the median, Bergstrand 1 in the tibial and Stewart and Copeland 1 in the obturator nerve. Cohn's case was interesting in that the growth recurred twice and with each recurrence took on a more sarcomatous appearance, the epithelial elements, which in the first specimen resembled glands, gradually becoming crowded out by the fusiform elements. The 2 cases that he reported in connection with this case are of less interest. Tumors of this type may also occur in serous membranes, Andrus having reported a tumor

in the pleura, diagnosed as neuroepithelioma, that was apparently non-malignant, and Mendl, one in the same membrane that was distinctly the reverse and metastasized widely. Marchand reported a tumor of this sort, in 1880, that was primary in the adrenal; at the time he was clearly puzzled as to its nature, but in a paper written eleven years later he formulated the diagnosis of "neurocytoma" for this growth. For some time it was confused with the neoplasm now known as sympathoblastoma.

SYMPATHOBLASTOMA

This corresponds in a way to medulloblastoma of the central nervous system as classified by Bailey and Cushing. It represents a step further along in the scale of development of the sympathetic system, but, paradoxically, shows less apparent differentiation. It tends to arise in the adrenal glands or in sympathetic chains elsewhere, or in the retina. Virchow is said to have recognized the tumor in 1864. Marchand's neurocytoma attracted attention to the group as a whole. Küster reported "two hitherto unknown tumors" of this group in 1905, interpreting them as glioma. Although pseudorosettes, which characterize this tumor type just as true rosettes mark sympathogonioma, were unheard of in glioma at that time, he nevertheless considered that the tumors he described represented a form of glioma. Wright was the first in America to take sympathoblastoma out of the class of lymphosarcoma, in 1910, calling it neuroblastoma, a name that has persisted to date in clinical circles. Sympathoblastoma is very malignant and tends to metastasize by one of two routes: If it arises on the right, it invades the liver and gives rise to the clinical "Pepper type." Should it arise on the left, it misses the portal system and metastasizes widely to the skeleton, particularly the skull—the clinical "Hutchinson type."

Sympathoblastoma is well discussed in Wahl's excellent paper, as also in the more recent article of Scott, Oliver and Oliver. If one considers the hypothetic "medulloblast" (a cell that was postulated by Bailey and Cushing as occupying a position midway in development between neuroepithelium and the neuroblast and spongioblast) one realizes that it carries three potentialities; it may differentiate into a functional nerve cell (neuroblast), into a supportive cell (spongioblast) or into a pigmented cell (pheochromoblast). In the first instance the ganglion cell is the ultimate product; in the second the ganglionic capsule cell or some other neuroglial cell, and in the third the pigmented pheochromocyte of such a gland as the carotid body, is the goal. It makes little difference whether these cells come to lie within the central nervous system or outside it. One not infrequently sees the three possible cell types combined in one tumor, as in those described by Martius, Cohn and others.

The existence of such a cell as the medulloblast is vehemently denied by many neurologists, but it seems that there must be a stage of this

type and that there are possibly cells which, reaching it, fail to develop further until later in postembryonal life. At all events this is Bailey's idea, and it is a useful one. Histologically, then, one may see specimens of sympathoblastoma that are largely composed of neuroblasts, truly neuroblastoma, while, on the other hand, there are those that show a fibrillary neuroglial architecture and more closely resemble glioma.

Lewis and Geschickter collected and studied 40 cases of sympathoblastoma, in 33 of which the growth arose in the adrenal medulla or in adjacent sympathetic ganglions. Landau reported a number of cases and reviewed the literature in 1912. He held that the identification of this type of neoplasm with glioma was not permissible. Reid reviewed the subject from the clinical-surgical standpoint. It may be seen from this brief review of the histology of the tumor that a number of subdivisions might easily be constructed, and Scott, Oliver and Oliver have done this. Bielschowsky has expressed the opinion that this is inadvisable as yet, since there are still so few facts to go by.

PHEOCHROMOBLASTOMA

Pheochromoblastoma develops from cells that originate about the periphery of the primitive nerve ganglions (hence "paraganglioma"). A great deal might be said about tumors of this description bringing up the topic of carcinoids of the intestine and appendix and the recently advanced theories of Hamperl on carcinoid of the bronchus, but, though neurogenous, such tumors do not seem to be entirely germane to this paper, and a short paragraph will have to do them justice. Masson has taken up the subject extensively in a paper of nearly 60 pages, wherein the topic of intestinal carcinoid will be found to have been most thoroughly discussed. To Kohn in 1905 goes the credit for first naming the paraganglioma.

GANGLIONEUROMA

Wahl has given a splendid résumé of the development of the present understanding of this tumor. Odier first recognized it in 1803; Virchow first classified it. The first well authenticated case was that of Loretz in 1870, to which Virchow referred; while Axel Key and Weichselbaum first described involvement of the adrenals by ganglioneuroma. Weichselbaum, in a paper of 4½ pages, proposed the name "neuroma," more specifically "ganglioneuroma" (*gangliöses Neurom*), for a tumor that he found in the adrenal. He attributed its presence there to a displaced fetal rest. His paper refers to reports of 6 other cases in the contemporary literature.

According to Wahl, there was then a gap in the literature until 1897, when Busse and Borst reported some cases, and Chiari and Knauss each reported others in 1898. Knauss held that the tumor originated

in perivascular nerve tissue. In 1934 Bergstrand attributed the same origin to a malignant tumor of the tibial nerve, thus completing the circle by reviving this thirty-six year old hypothesis. Verocay reported multiple ganglioneuroma as a "systemic disease." Bielschowsky now believes that ganglioneuroma stands somewhere between malformation and neoplasm and considers that the neurocellular components represent an early disturbance of embryonal develpoment.

While Geschickter justly rated ganglioneuroma as among the rarest neoplasms, McFarland and Sappington investigated reports of 143 cases which they listed. They commented on the composite appearance of ganglioneuroma, which contains ganglion cells, neurofibrillae and neuroglia. The variability of the ratios between these elements is well known and striking. Clegg and Moore, quoted by these authors, had reported the congenital appearance of some tumors diagnosed as ganglioneuroma and cited reports of 98 cases; 33 of the patients were under 10; 64 were over that age; 5 were over 60. Of 99 tumors of this type, 56 were in females and 43 in males. Ganglioneuroma may occur singly or show multiple incidence, particularly in connection with neurofibromatosis. Kredel and Beneke have been cited as reporting symmetric distribution. Montgomery and O'Leary reported a case in which the patient was literally studded with tumors of the skin diagnosed as multiple ganglioneuroma. A study of their cases raises some doubt as to its authenticity. Although the type cells of the tumors have an undeniably close resemblance to ganglion cells, they lie in a lymphoid reticulum rich in reticulin fibrils, and they are quite unaccompanied by capsule cells, neurofibrillae or neuroglia. A tumor in which ganglion cells are the sole components and in which the matrix is lymphoid is quite unheard of in the ganglioneuroma group. The tumors, had they been found in lymph nodes, might indicate a "shuffling" of embryonal tissue, particularly in the vicinity of the adrenals, but their presence in the subcutaneous tissue points strongly away from a diagnosis of ganglioneuroma.

Cushing and Wolbach described the occurrence of a paravertebral tumor, diagnosed ganglioneuroma, in a boy of 12, which was of itself in no way remarkable. One feature of the case, however, is most noteworthy, for the boy had had a biopsy specimen taken from a tumor in the same situation ten years before, and the diagnosis (made by Ewing) was "sarcoma, probably neurocytoma." Sections from this specimen were later scrutinized by Cushing and Wolbach, who agreed that the tumor was sympathoblastoma. The child was treated with erysipelas and prodigiosus toxins (Coley) and made a brilliant recovery. An operation later, to relieve paralytic symptoms, revealed ordinary ganglioneuroma, considerably scarred by fibrous tissue. Cushing and Wolbach postulated progression from a malignant embryonal tumor to well differentiated ganglioneuroma during these ten years.

MALIGNANT GANGLIONEUROMA

It is possibly not generally understood that ganglioneuroma may exhibit malignant forms in which it constitutes sympathetic neuroblastoma. This may be due to the fact that it sometimes shows great distortion of its usual architecture without metastasizing, as well as to the fact that in many instances well differentiated ganglioneuroma has been reported as metastasizing under circumstances in which the discriminating reader might suspect multicentric development and occurrence. This has complicated the matter.

Miller reported, in 1908, what was probably the first tumor of this group to be studied by means of silver impregnation. His paper refers to the literature up to that time. There were small tumors in the outlying lymph nodes, accompanying a larger tumor in an adrenal, interpreted by him as the primary growth. As all these tumors were well differentiated, one questions that they were malignant. In 1915 Robertson reported ganglioneuroma in the floor of the third ventricle, mentioned here to indicate that the growth may be central as well as peripheral. More important, he discussed 2 cases of what he termed "ganglioneuroblastoma." Both tumors showed immature as well as mature cells. One was "exquisitely malignant." Both were intra-abdominal, the first being near an adrenal and the second in front of the sacrum. Miller's article is well worth reading, as it gives an excellent review of the subject and a list of 71 references. Hoffman reported as ganglioneuroma a tumor that showed an admixture of immature neuroblasts, from which widespread metastases developed. Oberndorfer described a tumor that showed unequal differentiation of its elements, but as it failed to metastasize he reckoned it as nonmalignant ganglioneuroma. Von Fischer, writing later on this subject, classed Oberndorfer's tumor as malignant. Peters reported a similar tumor, which he regarded as nonmalignant, but von Fischer later included it in the malignant group. His criterion for a diagnosis of malignancy, which seems a sound one, is the presence of small undifferentiated cells in an otherwise differentiated ganglioneuroma. He added a tumor similar to the two just mentioned. Berner described malignant ganglioneuroma of the adrenal resulting in an addisonian symptom complex, a distinct rarity. The metastases he described were small and regional. One example of malignant ganglioneuroma has been observed at the New York Hospital. It was of the "hour-glass tumor" type described by Heuer and fulfilled von Fischer's criteria as to marked dedifferentiation, but failed to metastasize. In a long series of hour-glass tumors collected by Heuer from the literature, most were neurofibroma; 5 were ganglioneuroma; the rest were chiefly neurogenous, although a few connective tissue tumors were present. Finally, Harbitz reported malignant ganglioneuroma with widespread metastasis; his

article is in Norwegian, but there is a German résumé. It should be borne in mind that ganglioneuroma is distinctly a composite tumor that shows broad fields of neuroglia with definite nerve trunks coursing through them. The resulting teratoid appearance bears out the contention of Pick and Bielschowsky that ganglioneuroma represents a developmental defect rather than a neoplastic change. When it undergoes malignant change, however, the tumor characteristics are so marked that they cast doubt on this hypothesis. The finding is similar to the development of malignant characteristics in one group of cells in teratoma, as frequently seen in testicular teratoma and in mixed tumors of the parotid region.

FIBRILLARY NEUROMA

True Fibrillary Neuroma.—There appears to be some question as to whether or not there is a tumor that fully answers to this name. One must postulate a growth of peripheral nerve fibers that would form a tangle of nerve trunks in which there was little admixture of other nerve tissue. This it is difficult to do. Such a snarl of nerve fibers might be congenital; then it might represent a developmental defect or a prenatal trauma. Little tumors that come close to answering these specifications are often found on the dorsa of the hands, but again the question arises: Are they true tumors or merely proliferations of nerve fibrils in response to injury? Ewing in the last edition of his textbook devotes about fourteen lines to this form of tumor. In plexiform neurofibroma there may be overgrowth of nerve trunks "greatly in excess of that normally provided for the affected part," as Ziegler is said to have pointed out. An overgrowth of nerve trunks in composite neurofibroma, however, is not "true neuroma." It seems to me questionable that there is any such entity. The rare tumors involving large nerve trunks as illustrated in a case observed by Prudden (see page 479 of Ewing's book) may have only a superficial resemblance to true neuroma; in reality they represent neurofibroma that is rich in nerve fibers.

False Fibrillary Neuroma.—This is best illustrated by the familiar "amputation neuroma" that develops over the severed ends of the nerves of the stump after an amputation. Here, too, there is a great deal of fibrous and nerve sheath overgrowth, so that the tumors are rarely purely nerve trunk growths. It seems likely that the aforementioned small tumors of the dorsa of the hands, for instance, may be of a like nature, following injury to the much exposed nerve trunks of this region. Many tumors that appear to be composed of nerve trunks in ordinary stains will, on examination by silver impregnation, prove to contain no neuraxons, the apparent nerves being merely overgrowing empty sheaths.

Appendical Neuroma.—Masson has described as "appendical neuroma" a tumor-like proliferation of the myenteric plexus of the fibrotic appendix which includes an increase in the number of ganglion cells as well. It seems questionable whether such a development should be considered a tumor or merely a postinflammatory neural proliferation. It is fairly well known that the plexuses of Auerbach and Meissner often undergo marked proliferative enlargement in certain forms of chronic appendicitis, particularly in the subacute form sometimes known as "chronic ulcerative appendicitis," in which slight acute attacks follow rapidly on one another. This has been minutely described by Rabboni. Strangely enough, undoubted appendical neuroma has been observed only a few times in the course of careful examinations of some 2,000 appendixes at the New York Hospital. That the condition occurs is incontrovertible, but it seems to arise much more commonly in France and in the Latin populations than it does in a mixed population like that of the United States. It is frequently noted in Montreal, Canada. An excellent bibliography on chronic appendicitis will be found in an article by Beluffi, published in 1936.

NEURILEMOMA

One comes now to a consideration of the "specific nerve sheath tumor," as Stout has called it, the subject of more lively controversy than any other neoplasm of the nerves, just as the histogenesis of meningioma is probably the most controversial topic among those concerned with tumors of the brain. The two neoplasms are often associated, as pointed out by Cushing, Penfield and many other writers. They are likewise both frequent components of von Recklinghausen's neurofibromatosis. As the embryonal origin of both groups has not yet been quite firmly established, the reason for this controversy is evident; that which seems incontrovertible to one camp is lightly brushed aside by the other as being of no consequence.

Penfield and Mallory are the leaders of the one, Masson and Stout, of the other school. Penfield has maintained that these tumors are composed of modified fibroblasts, following the lead taken by Mallory some years ago in this connection. Masson and Stout, on the contrary, have supported the schwannian origin of these growths with equal enthusiasm, backed by a rather more numerous following. This controversy is well presented in a paper by Bailey and Herrmann (referred to in the introduction to this review) in which the views of Penfield are favored. It is a question not so much of the genesis of the Schwann cell as of how largely this cell figures in the formation of the tumor under consideration. Bailey and Herrmann expressed the opinion that little had been done to advance the matter by the writers of general papers on tumors of the peripheral nerves, but unfortunately they, too,

after building up quite a case for the fibroblastogenous theory, proceed to wreck it by saying: "What of the association of all these various pathological alterations? What of the undoubted fact that tumors of nerves have a definite structure not found in fibroblastomas elsewhere? What of the lesions in the brain?" What indeed? It is just here that one finds the kernel of the whole discussion. They suggested that nerves cause distinctive alterations in the appearance of fibroblasts, failing to remark that fibroblasts that form intracerebral scars after hemorrhage or softening do not undergo these alterations, any more than do those that form the connecting link in a severed nerve before lemmocytic proliferation begins. If one examines scars that involve nerves after trauma, one finds proliferation of the neurofibrillae, but no architecture reminiscent of neurilemoma. They ended on this note: "The origin of tumors of the peripheral nerves remains for us doubtful because cells of two possible sources (Schwann cells and endoneurium) form similar intercellular substances, and specific staining or impregnation methods for identifying their cytoplasm have not yet been devised." They accepted the term "neurilemoma" as proposed by Stout.

Having touched on the fringe of the controversy, one may now consider the history of the subject. These tumors were called "neuromas" by Virchow, who recognized them only as a specific form of acoustic nerve tumor. Verocay first described the bodies that are formed in these neoplasms and now bear his name. Because the tumors showed the presence of many parallel fibers, which he mistook for neuraxons, he called them "neurinomas," a name that has persisted to date. Antoni, in his 500 page monograph, published in 1920, divided them into two types: the A type, which forms Verocay bodies or whorls with rows of palisaded nuclei, and the B type (which he considered a degenerate A type), which forms rather myxomatous tumors that lack these more specific bodies and somewhat resemble sarcoma. Masson, in 1923, after exhaustive morphologic study of the tumor as it occurs in man, studied the experimental material sent to him by Nageotte and Guyon, in which segments of one sciatic nerve of the rabbit were transplanted into the sheath of the other, intact sciatic nerve. He believed that the tumor-like outgrowths at the ends of the implant were identical with the spontaneous neoplasms of human subjects. Using his trichrome staining technic, he came to the conclusion that the Schwann cells were specifically stained red, that they could thus be readily identified and that all the phenomena of the growth of the tumor were referable to the proliferation of these cells. The experiment was repeated by Bailey and Herrmann with great care, but they were not impressed by the specificity of their results.

In 1923 Erb reported 3 cases and discussed them at length, comparing others from the literature. He came to the conclusion that both

neurofibroma and neurilemoma are peripheral glioma. Borchardt, in an article that reflects only the Teutonic literature, accepted Bielschowsky's theory, which postulates that there is a faulty development of the sheath of Schwann, into which the connective tissue grows to form a neoplastic complex. He expressed the belief that the stimulus resides in the Schwann cell, the others playing sympathetic roles. As he put it: "Faulty gliotisation results in faulty mesenchymation."

In 1925 Krumbein and a little later Lauche (under whom he was studying) published elaborate articles on the mechanics of the formation of the Verocay bodies. They attributed the palisading of the nuclei to the relation of the cells to a central artery and said that this feature is not specific for neurogenous tumors as it is also seen in leiomyoma and sarcoma. Nestmann, however, noted that, although this was true, there was an encapsulated and organoid appearance to the Verocay body that was lacking in the other tumors. He expressed the belief that they were of schwannian origin and that the A type represented a more complete differentiation than the B type. This observation was borne out by the work of Murray, Stout and Bradley, whose article is to be quoted later on. Fittipaldi also espoused the schwannian theory and gave an excellent review of the literature, including many Italian sources. He opposed Penfield's views.

Stout followed Masson's lead with enthusiasm and has written one of the most comprehensive articles on the subject, with careful consideration of the microscopic and clinical aspects. Lhermitte and Guccione had claimed that victoria blue was specific for neuroglia and Schwann cells; Rhoads and Van Wagenen could not confirm this after careful observations carried out in Mallory's laboratory. During this time Masson had proposed the rather clumsy name "schwannoma" for these tumors, as the preferred term "peripheral glioma" had been preempted for another group. The name "lemmoma" came into brief use in a few papers, to be replaced by "neurilemoma," devised by Stout under the lexicographic scrutiny of the late Dr. Frank H. Vizetelly, at that time editor of the Funk and Wagnalls "New Standard Dictionary of the English Language." It will be noted that the word is "neurilemoma" and not "neurilemmoma," with two m's before the o.

Penfield revived Mallory's views on the fibroblastic origin of these tumors, largely on the basis that they form collagen and reticulin, a capacity which he denied for the lemmocytes and meningocytes. He said: "The telltale of tumor origin is the behavior of the neoplastic cell. . . . If the type cell produces collagen, elastic tissue and fibroglia fibrils the tumor must be considered fibroblastic." Rhoads and Van Wagenen made essentially the same statement, admitting that the fibroglia fibrils are often difficult to demonstrate. Bailey and Herrmann rejected the diagnostic value of these, as other cells form similar

structures that are usually best, or only, seen after fixation in Zenker's fluid. After all, the old dogma of the mechanistic production of fibers by specific cells has lost much of its pristine vigor.

In the same article Penfield described a "malignant schwannoma," the origin of which he wholeheartedly ascribed to Schwann cells, admitting in a footnote that he could not explain why these cells should form the collagen and reticulin fibers that were present. Geschickter carried out tissue cultures of neurogenous tumors and concluded: "The contention of Penfield and others that the subepidermal nodules are true neurofibromas and are different in origin from the palisaded neurinoma does not seem justifiable." He held that the palisading is dependent on the presence of myelin, in the absence of which it does not occur. Unfortunately, he rather hurried over the description of his tissue culture studies, which are not well documented in his article. He subdivided these tumors into neurilemoma and neurilemoblastoma, the latter somewhat conforming to the Antoni B type. Out of 850 neurogenous tumors which he studied, 400 were either one or the other of these; 240 were subepidermal. Of those considered as neurilemoma, 10 per cent were on acoustic nerves. The neurilemoblastoma, on the contrary, is usually subepidermal rather than subcutaneous or central. It is most frequently noted in children. Keller and Callender reported neurilemoma developing in the pericardial pleura (with typical Verocay bodies illustrated in the text). They called the tumor neurofibroma.

Tarlov examined 2 tumors of the vagus root, using Dockrill's silver impregnation, which he considered specific for lemmocytes, although he admitted that "it is not entirely reliable, but gives fair results in a high percentage of trials if the material is fresh and the staining is done within 6-24 hours after fixation." He examined 12 tumors diagnosed as neurilemoma without being able to demonstrate the presence of lemmocytes to his own satisfaction. He did find them in the neurofibroma of von Recklinghausen. These results he checked with Reich's toluidine blue method, finding numerous purple granules in the lemmocytes. He drew comparison between the lemmocytes and the oligodendroglia cells; the latter are not associated with collagen or reticulin, "which is a bit of indirect evidence against the Schwannian origin of perineural fibroblastoma." He finally concluded that the cells in neurilemoma that have been interpreted as peripheral neuroglia cells are in reality merely degenerated fibrocytes. The root tumors he described and studied he called "endoneurial fibroblastomas," which brings up an interesting point: They look as though they might be exactly that and they do not resemble neurilemoma so far as one can judge from the illustrations. Nobody can deny that there are such tumors as endoneurial and perineurial fibroblastoma, unless he denies the existence of endoneurium and perineurium. There must be a tumor of this sort,

a so-called "pure fibroma" of nerve. Nobody has, thus far, advanced a theory that the fibroblasts of these membranes may be racially different from those of ordinary connective tissue except to deny that they are offspring of the neural crest (Bailey and Herrmann). The point is well worth investigation.

Rather outdone by all this circuitous morphologic argument, Murray, Stout and Bradley made tissue cultures of embryonal human and rat nerve tissue to establish a standard tissue culture morphology for the lemmocyte in vitro. In human fetal spinal nerves the fibroblasts overgrew the lemmoblasts, but a 20 day rat fetus gave excellent cultures of the latter. Slow and sparse cultures resulted from explantation of human splanchnic nerve from a 16 year old girl, but they were relatively pure. The sciatic nerve of a 2½ month old rat showed simultaneously wallerian degeneration and outgrowth of both lemmoblasts and Remak cells. Myelin fragments were questionably phagocytosed by the former. Cells from experimental neurilemoma grew well, as did those from a human tumor of this sort. The excised Verocay bodies sent out the same type of cell in cultures, often resembling Remak cells. Explants from B type tumors were so liquefactive that cultures were difficult to maintain, but there was no evidence of degeneration as assumed by Antoni; rather the cells seemed to be vigorous and often underwent metamorphosis to the A type of growth. These cells all differed from the fibroblasts grown in the same cultures; they were more compact, were swollen at the middle and showed one or two wiry polar processes.

Ingebrigtsen had performed similar experiments with explanted rabbit sciatic nerve twenty-four years earlier, his observations agreeing with those of Murray, Stout and Bradley. He noted the outgrowth of Schwann cells from the severed ends of the segments and speculated as to why they had not been described in healing nerves in situ. Both Spielmeyer and Nageotte have described them in detail under these circumstances. Ingebrigtsen's beautiful illustrations bear a striking similarity to the photomicrographs of Murray, Stout and Bradley. Skinner, approaching the subject from the standpoint of morphologic histology, was able to distinguish two types of cells in tumors of acoustic nerves: the ordinary fibroblast and a smaller, plumper cell that he identified as the "neurilemma sheath cell." He expressed the belief that the fibroblasts are "defensive cells," which multiply in order to confine the tumor growth, with the result that a tumor of these two elements is formed. This is just the opposite of Borchardt's view.

As to site of occurrence, these tumors may be found in certain specified locations. Stout said: "The neurilemoma has not been found in every part of the body where there are nerves; instead it occurs commonly only in distinct localities, rarely in others and has never been found in the feet, genitourinary system, the lungs, the esophagus, the

rectum, etc." Its maximum size is about 6 cm. It is always encapsulated and pinkish yellow or pearl gray. With silver impregnations one notes that its silvered fibers have a wiry appearance and do not coil among the cells and wrap themselves about these but run a straight course tangential to the tumor cells. The palisaded Verocay bodies somewhat resemble pacchionian bodies, or arachnoid "caps"; they bear a resemblance also to the corpuscles of nerve terminals. The regular appearance of such structures in nerve tumors must indicate "consanguinity"; certainly they are not seen under other circumstances if one excepts the palisading noted in leiomyoma and sometimes sarcoma.

While Krumbein and Lauche used the palisaded nuclear architecture of muscular tumors to deny the specificity of the Verocay body for nerve tissue, Gosset, Bertrand and Loewy seem to have fallen into error through a too enthusiastic espousal of the theory that this nuclear arrangement always denotes nerve origin. They claimed that all the pedunculated tumors of the stomach hitherto supposed to be leiomyoma or leiomyosarcoma are, in reality, neurilemoma. Their illustrations appear to belie this hypothesis, as almost all the tumors shown resemble muscular neoplasm far more than they do neurilemoma.

Tumors of Antoni's B type are quite different in appearance; they lack Verocay bodies, and their cells run in all directions. The absence of bands of collagen and axis-cylinders and the presence of microcystic degeneration (possibly due to the liquefactive activity of the type cell) mark them off from ordinary neurofibroma. Foam cells are occasionally found in them, a point to be noted later in considering "neuroxanthoma."

Before proceeding to the next group of tumors, it would be well to mention a group that forms a sort of transition between neurilemoma and neurofibromatosis. Here both conditions are closely associated, with neurilemoma predominating. Turner and Gardner have reported observations on such tumors in 6 members of a single family in great detail and have extensively discussed the literature on the familial incidence of such tumors. All 6 patients had tumors of nerve sheaths or meninges, which followed a definite mendelian dominant trend in their incidence. The authors said:

> The first two cases are peculiar, in that, although both patients had well-advanced central neurofibromatosis, the involvement of the meninges by tumor was as marked as the involvement of the nerves by their specific growths. . . . Thus it may be that von Recklinghausen's disease should be regarded less as a specific disease process of the nerve sheaths (neurofibromatosis) and more in the light of the condition affecting all the sheaths and enveloping membranes of the central and peripheral nervous system.

Of 44 bilateral tumors of acoustic nerves which they collected, 37 were associated with von Recklinghausen's neurofibromatosis. There was a family of 38 members in which bilateral tumors of the acoustic nerves were transmitted as a mendelian dominant. They cited Roger,

Alliez and Sarradon as reporting a family in which bilateral tumor of the acoustic nerves was present in 1 member and cerebellar tumors in 4. Schaltenbrand reported a mother with bilateral tumor of the acoustic nerves and meningioma at innumerable points of the brain and cord; her daughter had a large meningeal tumor of the falx and neurilemoma of almost all the spinal nerve roots, with spongioblastoma in the upper part of the cord. Katzenstein, Kernohan and Parker, and Penfield and Young are cited as having reported similar cases. In the last-mentioned article a patient was described as having involvement of the brain, meninges, spinal nerve roots and sympathetic nervous system by neurofibroma, neurilemoma, meningioma and glioma. A number of nerve roots were involved, a tumor of each acoustic nerve was present, and the spinal cord contained several discrete tumors of astrocytic and ependymal types.

Miangolaira and Copeland reported the familial occurrence of neurilemoma; one tumor was in the sciatic, the other in the radial, nerve; Worster-Drought, Dickson and McMenemey cited 2 cases of central neurofibromatosis in both of which there were associated simple meningeal tumors. One patient had in addition 2 tumors of the cerebral cortex diagnosed as meningioangioma. Penfield and Young observed that among such cases there were many in which none of the tumors was of neurogenous origin, the type cell being really fibroblastic. The frequent occurrence of glioma in company with these tumors, however, constitutes a point that will bear careful consideration.

MALIGNANT NEURILEMOMA

One hears of "malignant schwannoma" or malignant neurilemoma. Stout has long been interested in tumors of this name, and Penfield described one at length, as already mentioned. Only a few others have been recognized and reported, for the reason that they are difficult to identify with ordinary hematoxylin-eosin technic, on which so many pathologists are still willing to stake their reputations. With a trichrome or tetrachrome technic one will find that malignant neurilemoma can be recognized by its failure to take up much of the green or blue (collagenophil) elements in the stain, whereas fibroma does so with avidity. Instead, malignant neurilemoma is predominantly reddish or pinkish in such sections, and its cells tend to be large, fusiform and vacuolated, resembling exaggerations of Masson's illustrations of the actively growing portions of his experimental neurilemoma in rabbits.

Such tumors may be very malignant and may metastasize. They tend to show a more or less peculiar type of growth with the development of coarse filiform processes on the type cells. Penfield stated emphatically that his tumor showed no palisading of the nuclei, although it did tend to form whorled cell complexes which, he stated, were quite

unlike Verocay bodies. Brandes reported one that he considered as "malignant neurinoma (Schwannoma)"; it showed epithelial elements which marked it as unusual. Bertrand and Bernard described a large fusiform tumor of the radial nerve that showed all the characteristics of malignant neurilemoma. They distinguished the type cell from a fibroblast by reason of its intercellular anastomoses and lack of any tendency to form collagen. Albot and Jehiel reported a "malignant glioma" of the cubital nerve that recurred four times; with each recurrence the type cell became increasingly metaplastic.

It is quite possible that more of tumors of this group will be reported as the use of Masson's trichrome technic increases and pathologists become more familiar with them. Many of the tumors have undoubtedly been diagnosed as spindle cell sarcoma in the past, and their true nature has been quite overlooked. A large and very malignant tumor of this sort was noted on the surgical service in the Cincinnati General Hospital several years ago, appearing as a terminal and fatal event in a case of neurofibromatosis. It had been diagnosed as "spindle cell sarcoma" until examined by the pathologist.

It is rather unfortunate that Geschickter has introduced the term "neurilemoblastoma" for one type of tumor in the nonmalignant group having origin specifically in nerve sheaths, the "subepidermal neurinoma," for the suffix "blastoma" usually indicates poor differentiation with malignant tendencies, and the tumor might therefore be confused with the malignant neurilemoma.

NEUROFIBROMA AND NEUROFIBROMATOSIS OF VON RECKLINGHAUSEN

This subject is not particularly controversial, the literature is voluminous, and one may pick up good articles on the type tumor almost anywhere. Lhermitte and Leroux (already cited in the previous section) pointed out possible questions that might be asked concerning neurofibroma: Does it contain Schwann cells or connective tissue or both? Is there such a neoplasm as pure fibroma of nerves? Is there such a neoplasm as primary sarcoma of nerves? Is neurosarcoma malignant neurofibroma? They examined a tumor classed as neurofibroma which had a normal nerve in its immediate vicinity, and they compared the two structures. Their conclusion was that the fibrous elements in the tumor were increased over those in the nerve. The answers to their questions are not forthcoming in their article with any degree of completeness, which tempts one to try to answer them. The consensus favors both fibrous tissue and schwannian tissue being present in neurofibroma. There seems to be no reason why there should not be such a neoplasm as pure fibroma of nerve, arising from endoneurium or perineurium or, for that matter, epineurium. In some instances neuro-

sarcoma appears to be true fibrosarcoma, while in others, as I have shown, it is malignant neurilemoma; that these conditions should be primary in a nerve is just as likely as that they should be primary in fascia or in any other connective tissue. In the course of their article the aforementioned authors indicated that they considered such lipid stains as sudan III and nile blue sulfate of value in identifying lemmocytes through the latter's contained lipids.

One feature that they do not stress and which still eludes one is the association of tumors of several categories in this one clinical entity neurofibromatosis. Besides neurofibroma, one sees neurilemoma, central glioma in various forms, meningioma and the like all occurring simultaneously in the nervous system, which recalls various theories concerning "systemic disease" and heredity. Worster-Drought, Dickson and McMenemey have discussed this feature from the standpoint of inductive embryology (their articles are cited in the previous section), suggesting that there is some inherent fault in Speman's "organizators" that arrange the architecture of the covering membranes during embryonal life. They have suggested that the disease should be known as "neuroblastomatosis," while the term "von Recklinghausen's disease" is reserved for the peripherally situated group of similar tumors.

Besides diversity in neurogenous tumors, in this disease, one observes apparently pure fibroma and lipoma associated with these and, to complicate matters, the strange hypertrophy of sebaceous glands known as "Pringle's disease," which may break down, undergo fibrosis and give rise to multiple keloid-like growths. Stewart and Copeland (see next section) have brought out clearly the difficulties that beset any one trying to make a coherent theory that will explain neurofibromatosis or the association with that disorder of these not strictly neurogenous tumors.

Hosoi, in an article dealing principally with malignant transformation in the tumors of von Recklinghausen's disease, presented, in 1931, an excellent review of the literature from the time of von Recklinghausen to 1930. He quoted von Fischer, who collected 466 instances of the syndrome, 299 in males and 167 in females. In 72 per cent of the subjects the disease developed in the third or fourth decade of life, the condition arising between the ages of 15 and 70. Its onset may be congenital or around the twentieth year. He mentioned the puzzling complication Pringle's disease. Levin and also Tucker expressed the belief that neurofibromatosis may represent an endocrine dysfunction, but this was denied by Schneiderman. In about 13 per cent of the cases one or more of the tumors undergo malignant transformation.

Neurofibroma may occur subterminally, near the nerve endings, and be intimately connected with the overlying skin. The tumors of this classification may be confused with tumors of other categories, as myxoid

neurinoma or "neurilemoblastoma" (Geschickter) as well as with a type of xanthoma concerning which more will be said later. Only by careful study with specific nerve stains and impregnations is one able to differentiate between them. They range from small growths, largely fibrous and containing elements other than the nerve sheath, such as nerve trunks, through the myxoid tumor, which may exhibit foam cells, to the bright sulfur yellow "neuroxanthoma." They may occur anywhere along the course of peripheral nerves, arising singly or as multiple growths. They may be small, or they may reach the enormous proportions of plexiform neurofibroma.

The latter was first described at length by Bruns, in 1870, who named it *Rankenneurom*. *Ranken* denotes shoots or tendrils in German. Verneuil called it "plexiform neuroma," which is a term still much in use. It is most frequently seen on the head, where the temple is a favorite site and where it may undergo malignant degeneration. Bruns, considering it a sort of elephantiasis, called it, in a subtitle, "elephantiasis neuromatodes," and Pick showed that neurofibroma is sometimes associated with local hypertrophy of organs, particularly when it occurs in the alimentary tract. In 7 of Bruns's cases it was familial and apparently hereditary. The skin over it may be pigmented, and in an instance studied at the New York Hospital, in which a tumor diagnosed plexiform neurofibroma arose over the sacral region, there was extensive involvement of the cutaneous tissue and subcutaneous fat by melanoma, which was of an infiltrating, though apparently nonmalignant, type and followed the vessels in its distribution.

Neurofibroma in the form of multiple small tumors may occur during pregnancy, according to Brickner, who described this condition in 1906, naming it "fibroma molluscum gravidarum." The tumors regress and disappear entirely with the termination of the pregnancy. Since that time the condition has been occasionally noted in obstetric practice. Bricker's illustrations show small tumors with rather insignificant cores and very thick epithelium, which raises the question as to whether they are not more like verrucae than fibroma.

Sometimes neurofibroma shows groups of pigmented cells in close association with the nerve bundles or pseudonerves, a fact that affords a connecting link between it and melanoma, a point that Masson was quick to note and follow up, as will be explained later. As one may see, it is quite possible that many if not all the tumors regarded as "true fibrillary neuroma" may not really belong in the group classed as well differentiated neurofibroma, and it will be remembered that Virchow insisted that in all his cases of fibrillary neuroma the growths were "complex organoid tumors," a definition that fits neurofibroma perfectly. The tumors of this group may contain nerves with sheaths, nerves without sheaths, sheaths without nerves (containing Schwann cells) and about all this masses of fibrous tissue.

NEUROGENOUS SARCOMA

This neoplasm has been called "neurogenic sarcoma" by Ewing, Stewart and Copeland, Quick and Cutler and by other writers. It seems that it would be better to follow the lead of Dorland's "American Illustrated Medical Dictionary," which has made a beginning by defining "neurogenic" as implying the production of nerve tissue and "neurogenous" as denoting something derived from that tissue. Schultz has used this term in the title of his general paper on neurogenous tumors. The use of "neurogenous" for these tumors is therefore proposed, after written consultation with Mr. Charles E. Funk, editor of the "New Standard Dictionary of the English Language," and personal conversation with Dr. Ewing. This proposal is admittedly arbitrary, but it would be better to divide the synonyms and apply "neurogenic" to nerve-producing factors or activities and "neurogenous" to that which is produced from or by nerves. This sort of arbitary decision has proved valuable in connection with "tubercular" and "tuberculous," which are now seldom confused except by the laity.

Quick and Cutler published an article on the subcutaneous form of neurogenous sarcoma in 1929; Stewart and Copeland followed this, in 1931, with an 85 page paper in which most of the subject matter of neurofibromatosis is reviewed in a discussion of so-called "neurogenic sarcoma." They have tabulated the data on 64 cases observed at the Memorial Hospital in New York and appended a tabular review of others collected from the literature. While they do not indicate that there was extensive study of these tumors by means of special staining methods, they are able to make most of their points clear on the basis of hematoxylin-eosin staining. Charache has recently studied 19 neurogenous tumors diagnosed as sarcoma, finding 11 in the extremities, 2 in the scapular region, 2 in the axilla, 2 in the gluteal fold, 1 in the abdominal wall and 1 in the ear. This corresponds closely with my experience at the New York Hospital. Eight of the 11 tumors Charache observed on the extremities were on the thigh; 57.9 per cent of the patients had from one to six excisions and as many recurrences; 4 showed pulmonary metastasis, retroperitoneal in 3 and cerebral in 1. In 11 patients the condition ended fatally; 8 patients were alive at the time of publication; of the ones who died, 6 had tumors of the thigh. The thigh seems to be a favorite site for neurogenous tumors.

Neurogenous sarcoma has been recognized for a long time. Garré studied a series of cases of neurofibromatosis in 1892, in which one or more of the tumors showed malignant changes. His work was instigated by Bruns, who expressed the opinion that in a large number of cases of the latter type the malignant change might originate in plexiform neurofibroma. Garré quoted Courvoisier, who found that in 53 of 600

instances neurofibroma became malignant. In 15 of these, the malignant neurofibroma was "pure sarcoma" and in the rest, sarcoma mixed with myxomatous or other connective tissue elements. It is interesting that one of Garré's malignant tumors showed epithelial cysts, not unlike those described earlier in this review.

Stout recognized two types of malignant tumors in nerves: (*a*) those with the morphologic characteristics of sarcoma and (*b*) those that have neuroepithelial admixtures. The former are usually seen as terminal phenomena in neurofibromatosis and are seldom found on the torso, a supraclavicular or postcrural distribution being, according to him, the most usual. They may be of two origins: the fibrous tissue of the nerves and the neurilemma. The latter type has already been discussed. The former may arise in the fibrous neurilemma and are therefore essentially fibrosarcoma. One might add that there are two possibilities here: fibrosarcoma and retothelial (reticulum cell) sarcoma, as both fibrous and reticular tissue are present.

Stout said: "The Memorial Hospital school, headed by Ewing, have come to believe that the majority, if not all, of the malignant spindle cell sarcomas of the skin and deeper tissues of limbs and trunk arise from peripheral nerves." This conception first appears in the second edition of Ewing's "Neoplastic Diseases." He then traced an increasing positiveness in the statements of this author in the succeeding editions until, in the third the following statement appeared: "The great majority of spindle-cell sarcomas of the skin are of neurogenic origin and should be classed with neurofibroma, but it is probable that in comparatively rare cases tumors arise from other structures not connected with the nerve trunks or filaments." Stout expressed the belief that, as von Recklinghausen's disease was absent in most of the cases reported by Quick and Cutler, Stewart and Copeland and other members of the Memorial Hospital School, and as he could find no microscopic description that indicated the presence or absence of nerves, their conclusions were unwarranted. He said: "In none of these articles can the present writer find any microscopic details, criteria of differentiation from other tumors, or any evidence to support the hypothesis that all, or even a large percentage, of spindle-cell tumors are 'neurogenic' sarcomas."

However this may be, Ewing has replied as follows in a discussion printed at the end of a paper by Adair and McLean (cited in the first section of this paper): "I am willing to make the diagnosis of neurogenic sarcoma and support the diagnosis against criticism. In this field I am not at all a pioneer, and do not occupy an isolated position as has sometimes been suggested." He pointed out that the older German literature cleared up this matter many years ago. If he had made any contributions on the subject, he remarked, it had been by way of calling attention to these neoplasms.

The diagnoses of the Memorial Hospital school are usually based on observation of a certain "curly" arrangement of the fibers of these tumors, which run in interlacing bundles and sometimes radiate in a stellate fashion from rather indefinite centers. Ordinary fibroma tends to have a more parallel arrangement of its fibers. If one impregnates these curly tumors by the Nonidez modification of the Cajal silver nitrate block method, one is able to demonstrate nerve fibers in most of them. Another point on which the two schools may be talking at cross purposes is the use of the term "neurogenic"; the one may take it to mean "derived from nerve tissue," while the other reads it "derived from those tissues which together go to make up a nerve trunk." As often as not the last idea is valid, and the use of "neurogenic (better "neurogenous") in this sense is convenient. In other words, when one speaks of "electric wire," one usually infers the presence of silk, cotton and paraffin insulating material, without specifically mentioning it.

As Stewart and Copeland pointed out, it is possible to recognize various types of neurogenous sarcomas, which they arrange in grades according to Broders' plan, but to grade a tumor is to give it a prognosis, not a new classification. Aside from differentiating between those tumors that arise from neurilemma proper and those that have a reticular nature (arising from endoneurium or perineurium) and differentiating these from the neoplasms that arise in epineurium and are therefore ordinary fibrosarcoma, not much can be done to subdivide them. Those who believe that collagen and reticulin are the same chemical substance will not be impressed by this subdivision.

Neurogenous sarcoma is notably radioresistant; furthermore, it may begin as innocent-looking "fibroma," only to recur and with each recurrence to become more malignant until it metastasizes. Bick has analyzed a series of 24 cases of fibrosarcoma; 2 of the tumors arose from nerve sheaths. He found that it makes little difference from what tissue fibrosarcoma arises, nerve or otherwise—it is imperative to take drastic steps like wide excision or amputation after one recurrence. The follow-up histories on the patients showed that the best results followed radical surgical intervention.

TUMORS OF NERVE TERMINALS

Early in this review the various forms of nerve endings and end organs were briefly described. That there should be tumors of these is to be expected. Ewing has mentioned peculiar tumors that he has seen in muscle that did not seem to be of muscular composition. He interpreted these as possibly tumors of motor end plates. In the section on neuroma the questionable status of "tumors" composed of cell processes was mentioned. That there should be tumors comprised of

cells that form the adnexa of such organs as the Wagner-Meissner and Ruffini corpuscles is another matter, for these have numerous cells associated with the arborizing nerve endings, cells that are supposed to be modified lemmocytes. A very common tumor, possibly more developmental defect than true neoplasm, is the pigmented mole or nevus. This may attain great size and undergo malignant change to produce one of the most vicious and widely metastasizing tumors in the long list. For this reason one may consider pigmented nevi as tumors for the purposes of this review.

The old idea was that they were a product of infiltration (Miescher called it *Abtröpfeln,* or trickling down) of pigmented cells from the basal layer of the epidermis into the pars papillaris, where they congregated and grew into tumors. It was early recognized that some of them appeared to be composed of connective tissue melanophores, while others retained an epithelial aspect and for this reason were known sometimes as "melanosarcoma" and again as "melanocarcinoma." This theory held up to comparatively recent time, certainly as late as 1930.

Soldan, in 1899, called attention to the association of such growths with von Recklinghausen's disease, but his article was not incisive and attracted little notice until it was excavated by Masson after he had independently come to the conclusion that these tumors were really neurogenous.

He had noted a tendency of their cells not only to form nests beneath the epidermis but to produce peculiar corpuscular structures that were particularly prominent in moles of the scalp and which he named *lames foliacées.* He published three articles in 1926 which immediately attracted universal attention and provoked much discussion that stimulated further investigation. Prior to Masson's discovery of these bodies, Dawson had written a monograph that covered all the varieties of nevus and presented a long list of references. Miescher had also gone into the subject extensively in connection with the so-called dopa (dioxyphenylalanine) reaction for the demonstration of immature melanin. He opposed Masson's views for some time with considerable force but was finally won over to them. Becker has written three articles that demonstrate the gradual shift toward Masson's views, as they cover the period from 1930 to 1934 during which these views were being put forward.

Briefly summed up, Masson's idea is this: The *lames foliacées* are distorted Wagner-Meissner corpuscles in which the modified Schwann cells of the organ play the dominant part (hence the subtitle "Schwannoma" in one of Becker's papers). After this idea had found favor in France, it was accepted by Ewing, who gave it wide publicity in a number of articles and in his textbook. I undertook a number of investigations with silver impregnations in an attempt to demonstrate

nerve endings in the cell nests and *lames foliacées*, and while these were moderately successful, they were much inferior to those of Laidlaw and Murray, who used the Rogers technic of silver impregnation. Once the theory was established, it became evident that some of the pigmented nevi showed more definite nerve bundles and less definite cell nests, which led to their being called "neuronevi." They have a striking resemblance to pigmented neurofibroma; it is possible that they take origin a little higher up the sensory nerve than do their more terminally situated relatives. The pigment in these is a variable component, being copious in some and rare or entirely absent in others (which gives rise to the rather ridiculous term "amelanotic melanoma"). Masson attributed this variability to fluctuations in the melanin metabolism of the cells.

Any connection between these tumors and end organs other than the Wagner-Meissner corpuscles is yet to be discovered, although it seems not unlikely that such an interrelationship may be found at some time in the near future. Masson expressed the belief that the tumors classed as intradermal melanoma arise at the very tips of the tactile nerves in the Merkel-Ranvier cells that are intercalated among those of the basal layer of the epidermis. Ewing cited Thoma as describing a painful tumor apparently composed of distorted pacinian corpuscles; this is a little suggestive of the glomus tumors to be discussed later.

Malignant melanoma tends to show much less propensity to form laminated bodies, as might be expected in a more lawless growth. Here again the amount of melanin varies; one may see such tumors showing much pigment in the primary growth and none at all in some of its metastases.

There are numerous subvarieties of melanoma (see Dawson): intracutaneous (pigmented spots), mongolian spot, papillary, verrucose (often associated with verruca vulgaris), subcutaneous and the like. As they are all variations on the same theme, there is no need for describing them at length. The enormous "bathing trunk nevus" is the largest variety, corresponding to its name in distribution and size. It may be associated with neurofibromatosis or with multiple large nevi all over the body. It is a congenital involvement. In an instance described elsewhere in this paper a nevus arose over the sacrum and resembled pigmented plexiform neuroma, hanging down like an apron. There was deep penetration of the underlying tissue by pigmented cells that followed the course of the vessels.

The appearance of melanoma in the meninges of the brain and cord has been the subject of a number of articles that are listed in the bibliography. As the tumors described did not arise in peripheral nerves, comment on them will be omitted here.

Before leaving the subject it may be pointed out that melanoma forms corpuscular, sometimes whorled, bodies; neurilemoma produces somewhat similar structures, the Verocay bodies, and the purest form of meningioma is characterized by epithelioid masses of a whorled architecture. This must mean something, occurring as it does in the cells of the covering membranes of the nervous system, and it should suggest "consanguinity" of these neoplasms. The proponents of the fibroblastic origin of such whorls in the meninges and neurilemma have as yet withheld their fire from melanoma.

NEUROXANTHOMA

Recently I published an article in which it is proposed that a certain form of cutaneous xanthoma be included among the neurogenous tumors. Silver impregnations demonstrate nonmyelinated nerve fibrils in this type of growth; pigmented spots or actual melanoma often cover it, being interposed between it and the epidermis and seeming to merge imperceptibly with the xanthoma. The structure of such a tumor is almost identical with that of Ewing's "neurogenic sarcoma" except that the cells are well differentiated and resemble adult fibroblasts, with foam cells interspersed. They are not malignant. It is suggested that these foam cells, which contain the bright sulfur yellow pigment that characterizes the tumor, are of schwannian origin rather than ordinary lipophages, such as are seen in the xanthoma and xanthelasma of lipoid dyscrasias and of senile atrophy. This hypothesis is far from being proved, but there is much in its favor, as foam cells occur in connection with neurofibroma and neurilemoma. The article in which the theory was advanced is still too recent to have attracted attention or aroused the discussion that is ultimately hoped for.

Neuroxanthoma almost invariably arises on the skin of the thigh (like other neurogenous tumors already mentioned); it is sulfur yellow on the section surface, well encapsulated and often unsuspected until incised, being mistaken for hard fibroma. The consistence in situ is very firm and somewhat rubbery. That it is different from Geschickter's "neurilemoblastoma" is indicated by the color, the fact that it arises in adults rather than in children and that it does not resemble the tumor he described histologically. Occasionally one finds foreign body giant cells, as one does in the apparently related melanoma. The reason for their presence is not evident; Masson's explanation (applied to melanoma) is so succinct that one cannot help quoting it: *"Les cellules de Langerhans sont."* He thus admitted his inability to explain their presence adequately.

GLOMIC TUMORS

The glomic (or glomal) tumors are rather recent arrivals in the company of human neoplasms. Stout, in an interesting article on this

subject, gave a good résumé of the development of knowledge concerning them. With his usual industry, he traced their clinical history, or recognition, back to Morgagni (who quoted Valsalva's comment on one) as well as to other early investigators of the middle of the eighteenth century, who knew them simply as small, very painful tumors. They were first arrayed with the tumors diagnosed as angiosarcoma, until Müller changed the name to "perithelioma." They were, however, reported as angiosarcoma as late as 1927 by Carstensen (see Stout).

The recognition of their true origin in hitherto undescribed organs was the outcome of a case described by Barré in 1920, followed by a report of 3 more in 1922. Meanwhile he had turned over 2 specimens to Masson for study, with the result that this investigator, who had already worked on a case in 1916, discovered that the arteriovenous complexes of which these tumors were composed were intimately connected with complicated networks of neurofibrils. There were prominent Vater-Pacini corpuscles flattened out over the capsules of these tumors which might account for their painfulness. Believing that the beautiful organization of the tumor indicated its derivation from an organelle, he devoted much time to an investigation of the subcutaneous and subungual tissues of the body and discovered that there were, indeed, small neurovascular organs ("neuromyo-arterial glomi"), to which he attributed some sort of manometric control over the peripheral circulation. The frequency of the occurrence of this man's name in this review cannot but strike the reader and prove how valuable have been his researches in this field.

The importance of this discovery was immediately recognized, and reports of cases and of investigations began to appear in the literature. Popoff carried out extensive and meticulous histologic investigations on the glomus in health and in inflammation, and Masson continued to establish his theories more firmly. Stout published a graphic and interesting review of the development of this phase of tumor study, with a long bibliography. In a later article, Masson traced the history of the glomuses back to 1837, when Berres first noted them in erectile organs. In 1844 J. Müller was the first to describe them accurately. Masson modestly noted: "In 1924 and 1926 P. Masson, attracted to a study of these anastomoses by the tumors that arose from them, showed that they are included in a special connective tissue group, that they possess an extremely rich nervous apparatus, and he gave to this vasculonervous ensemble the name of neuromyoarterial glomus. He followed this by describing their pathology." Appended to his article (1935) there is a list of 58 references to the literature.

Barré and Masson, in 1924, published a joint paper in which they noted that the glomuses and Ruffini's corpuscles might be identical. In 1928 Greig published an article on the painful tumors of the glomuses,

tabulating data obtained in a review of 23 cases, including 3 of his own. In 1934 Adair reported on tumors of the glomuses from the clinical standpoint in a brief and pithy paper. The first such tumor that he encountered was undiagnosed until the sections were seen by Ewing, who recognized their nature from Masson's description. Since then Adair has collected 10 glomal tumors. Bailey discussed the glomal tumors in 1935 and suggested that they be called "glomangioma." He expressed the belief that a close analogy may be drawn between these and the pigmented nevi, as both represent hypertrophied end organs. Masson again worked on them in 1936, focusing his attention on the nerve elements of their makeup. He mentioned that E. R. and E. L. Clark were observing the action of these elements in their experimental "window cultures" in rabbits' ears. Radasch published a comprehensive article with a good bibliography in 1937, in which he tabulated data on 90 cases from the literature.

It is because of their very rich supply of myelinated and nonmyelinated nerves, with numerous interstitial branching end organs, that these tumors have been classed with those of the peripheral nerves. It may be that this is a mistake and that they should fall into the family of hemangioma, in which one may demonstrate a very rich nerve supply by means of silver impregnation. However, it may be as well to keep them in the category of neurogenous tumors until they are thoroughly understood.

Histologically speaking, the glomus is composed of small vessels with disproportionately thick walls that are arranged in complex congeries in which there is free interchange between the arterial and venous blood flow. Neurofibrils surround these in a "raveled sock" network of loops and coils. The end organs of these are situated in the stroma of the organ; they are branching, and their filaments terminate in buds somewhat like those of the tactile nerve endings. The tumors that arise from these organelles show a marked increase in the number and size of the vessels, a certain disorder of these, and increased thickening of the walls, which are composed of layers of epithelioid cells that contain no myofibrils but are apparently altered muscle cells, concerning the nature of which there is considerable speculation and dispute. No malignant analogues of these tumors have as yet been described.

From the clinical standpoint, they are most frequently found under the finger nails but may occur anywhere that glomuses are present, usually on the extremities. They vary from 1 to 3 cm. in diameter and from red through purple to blue, which is their usual color. They are characterized by the exquisite pain that is caused, usually spasmodic in character and described by the patient as "burning, piercing, or bursting." A paroxysm may be set off by the pressure of clothing or by so light a stimulus as the falling of a sheet of newspaper onto the surface

of the tumor. The tumor is said to occur more frequently among Jews than among Gentiles, which distinguishes it from the painful dermatomyoma, with regard to which no racial predisposition is noted. Sex variations are puzzling: Stout remarked that, of the subungual tumors that he had observed, 17 were in females and only 2 in males; of the tumors that were located elsewhere in the body, however, 14 were in males and only 6 in females. He reviewed 62 authentic cases. That such tumors are uncommon is attested to by the fact that only one has been submitted to the laboratory of the New York Hospital in the course of the past eight years, in spite of a very lively outpatients' department service and expectant watchfulness on the part of the laboratory staff.

SUBCUTANEOUS LEIOMYOMA

Stout included tumors of this description in his classification of neoplasms of peripheral nerves because of their painfulness and because of a composition somewhat similar to that of glomus tumors; they lack the elaborate vascular structures of the latter, however. In his article on this subject Stout notes that the first subcutaneous leiomyoma was described by Axel Key in 1873. It had been removed from the finger of the poet Stranberg. In 1884 Malherve reported 5 solitary painful growths diagnosed as leiomyoma. Babes in the same year classified such tumors according to their origin from vessel walls, from arrectores pilorum, from remnants of the panniculus carnosus and, lastly, from misplaced embryonal muscular rests.

The distribution of these growths over the body is interesting and bears out Babes' classification; they occur on the extremities, face, nipples and genitalia, where smooth muscle may be rather abundantly present. While multiple dermatomyoma is more common in males than in females, the occurrence of the solitary growth is about the same in both sexes. Of 87 patients with the multiple variety, 78 per cent showed development of the condition before the age of 30; of the 66 solitary tumors collected by Stout, only 47 per cent were observed before that age was reached. There is no racial difference in their occurrence.

A perusal of Stout's paper will convince the reader of a certain similarity of these tumors to those of the glomuses. They tend to be very vascular, but the vessels are not arranged in an orderly fashion. Most investigators have been unable to demonstrate much nerve tissue in them, Stout included, but Grzybowski was successful in demonstrating large numbers of neurofibrils that ran parallel with the smooth muscle fibers by using a modification of the Cajal silver nitrate impregnation. The fibrils often penetrated the muscle bundles deeply and formed their central axis.

It will be noted that Stout drew a distinction between multiple dermatomyoma and solitary, painful leiomyoma of the subcutaneous

tissue. Considerable space has been allotted to these neoplasms in this review, as there is nothing much to be found concerning them in the average textbook. As Stout remarked:

"The existence of the solitary superficial leiomyoma cannot be very well known to the English-speaking public, since only three proved cases have been published in that language." He had the opportunity of studying the condition in 15 patients; at the New York Hospital my colleagues and I have seen but 2 in an eight year period. In Stout's opinion this group of tumors, together with most of the glomus tumors, forms the bulk of painful subcutaneous tubercles, "tubercula dolorosa."

MISCELLANEOUS TUMORS

For the sake of completeness those tumors closely associated with nerve trunks should be mentioned. Near these are collagenous, adipose and vascular tissues, two of them forming a sort of accessory insulation, and it is quite possible to have fibroma, fibrosarcoma, angioma, lipoma, myxoma and the like arising from the outer portion of the epineurium, or just outside of it in the adjacent tissue. The frequency of such apparently adventitious growths in neurofibromatosis has already been discussed in this review, and it seems unnecessary, therefore, to take up such tumors in detail, as they do not differ from those found elsewhere in the body.

RECAPITULATION

An attempt has been made in this review to discuss the various tumors of peripheral nerves with special emphasis on their histologic character. In so doing, those points that have given rise to controversy have been particularly stressed and presented to the reader not only for his information but in order to afford him an opportunity to form a personal judgment of the merits of the arguments on both sides. It is not easy to maintain an entirely objective attitude under controversial circumstances, but I have tried not to express personal opinions any more than seemed unavoidable. If in spite of this the reader finds the presentation biased, he can always turn to the original articles listed in the bibliographies for the views of either side in the controversy.

After reading this review it will be found that neurilemoma (alias: neuronima, perineural fibroblastoma, schwannoma, peripheral glioma) and neurogenous sarcoma are probably the most controversial subjects touched on. The inadvisability of considering "fibrillary neuroma" as a true tumor has been discussed and a classification devised to take care of it provisionally. Tumors closely associated with the peripheral nervous system but not arising directly within it have been introduced into the review for several reasons: They show close association with nerves, they are painful, they present a relative novelty, not being

readily found described outside of articles in medical magazines—and, lastly, they are included because other writers on this subject have also included them. One of them, the glomus tumor, has been recognized only within the last decade or so, and its status is still in doubt. Is it primarily of nervous or vascular origin?

It is hoped that this article may assist in clarifying the subject for the reader or, failing in this, in stimulating his further effort to clear it up for others. The bibliographies have been purposely kept as compact as possible, although they could readily be made twice as voluminous. Authors whose articles have not been cited may, therefore, understand that these were either unfortunately overlooked or mention of them reluctantly omitted through lack of space.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—David Riesman, professor of the history of medicine in the graduate school of medicine of the University of Pennsylvania, died on June 3, 73 years old. He became associated with the university in 1908 and was professor of clinical medicine from 1912 to 1933. He was a prolific writer in the fields of pathology, medicine and medical history. He was an editor of the "American Textbook of Pathology."

David Perla, associate pathologist and immunologist at Montefiore Hospital, New York, died June 14, of a heart attack, at the age of 40.

Stanhope Bayne-Jones has resigned as dean of the Yale University school of medicine. Francis G. Blake, Sterling professor of medicine, has been appointed acting dean.

Giuseppe Sanarelli, Italian bacteriologist of the Pasteur school and discoverer of the virus of rabbit myxomatosis, has died at the age of 76 years.

Warren H. Lewis, who has retired as research associate in the department of embryology of the Carnegie Institution of Washington and as professor of physiologic anatomy in the Johns Hopkins University, has become a member of the Wistar Institute of Anatomy and Biology, Philadelphia. The Carnegie Institution is maintaining at the Wistar Institute the program of Mrs. Warren H. Lewis, research associate in the institution, on the same basis as before in Baltimore, and the International Cancer Research Foundation is continuing its grant for research assistance to Dr. and Mrs. Lewis as in past years.

Society News.—The 1940 Graduate Fortnight of the New York Academy of Medicine will be held October 14 to 25. The subject is "Infections." A carefully integrated program will be presented of clinics, discussions, demonstrations, addresses and exhibits. For information write to Dr. Mahlon Ashford, 2 East One Hundred and Third Street, New York.

The 1940 annual meeting of the Society of American Bacteriologists will be held at the Hotel Jefferson, St. Louis, December 27-29.

A. V. St. George is the new president of the American Society of Clinical Pathologists; J. L. Lattimore is the president-elect, and A. S. Giordano is secretary-treasurer.

The American Association of the History of Medicine has elected Esmond R. Long president and reelected Henry E. Sigerist secretary. The next annual meeting is scheduled for Atlantic City, May 4-6, 1941.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Experimental Endocarditis Lenta. W. J. MacNeal, J. J. Spence and M. Wasseen, Am. J. Path. **15**:695, 1939.

By repeated intravenous injections of large amounts of pure cultures of isolated streptococci in serum broth it has been possible to transmit endocarditis lenta of man to rabbits. The endocardial lesions in the rabbits have usually been quite large and easily recognizable on gross inspection. Microscopically, the lesions resemble those of the human disease. Large colonies of the streptococci are easily found. In the rabbit there is sometimes an evident tendency for these lesions to heal, which suggests that this animal may be of value in testing therapeutic measures against the disease. From Authors' Summary.

Experimental Rickets in Rats. G. S. Dodds and H. C. Cameron, Am. J. Path. **15**:723, 1939.

The cartilage remnants in the rachitic metaphyses of experimental rats were observed to behave as follows: Considerable osteoid is deposited on the surfaces of the cartilage remnants—osteoid envelopment of cartilage. Osteoid is deposited in the opened lacunae near the surface of these cartilage remnants by the action of osteoblasts, which enter the opened lacunae from the adjacent marrow—osteoid infiltration of cartilage. Cartilage cells farther below the surface of the remnants undergo rejuvenation; many of them divide mitotically and assume the form of osteocytes. While this change is going on, uncalcified osteoid matrix is deposited about them in the unopened lacunae—internal osteoid reorganization of cartilage. In the foregoing internal reorganization process the cartilage cells undergo metaplasia into an osteocyte-like form, but the matrix of the cartilage is not changed. It persists, to become calcified during the healing of rickets and to serve as a base for the deposition of bone in the formation of the trabeculae of the restored bone. From Authors' Summary.

Effect of Yeast on Cirrhosis Produced by Lead Arsenate. W. C. Von Glahn and F. B. Flinn, Am. J. Path. **15**:771, 1939.

The incidence in rabbits of hepatic cirrhosis produced by ingestion of lead arsenate is reduced when powdered brewer's yeast is added to the diet. There is no apparent relation between the amount of hepatic glycogen and the quantity of arsenic in the liver, nor is there any obvious connection between the glycogen content of the liver and the incidence of cirrhosis. From Authors' Summary.

Disruption of Circulation of Bone. C. Huggins and E. Wiege, Ann. Surg. **110**:940, 1939.

Surgical interruption of the principal nutrient artery and vein of a bone in the rabbit was always followed by necrosis of the bone marrow. The amount of necrosis varied in extent; the central marrow and a part of the peripheral marrow adjacent to the bone were always involved. There was total absence of inflammatory exudate. An infiltrating type of repair by new capillaries and macrophages arising from the living marrow at the periphery of the necrotic zone was apparent four days after ligation, and no necrotic masses remained after twenty-two days.

Only slight evidence of removal of the necrosis by phagocytosis was obtained. The earlier repair of the marrow was characterized by loose isolated cells in an edematous gelatinous marrow, but after seventy days the marrow was indistinguishable from normal. The absence of scar tissue in the reaction to the necrosis was striking.

FROM AUTHORS' SUMMARY.

REACTIONS OF THE MOUSE UTERUS TO OVARIECTOMY. E. HOWARD, Bull. Johns Hopkins Hosp. **55**:341, 1939.

There is no marked qualitative difference in the degree of uterine involution after ovariectomy in mice with or without the adrenal X zone, nor is there any evident difference between mice and rats with respect to this uterine reaction. Thus the association between the presence of the X zone and a stimulation of certain male characters has not been found to be supplemented by a suppression of female differentiations.

FROM AUTHOR'S SUMMARY.

THE RETICULO-ENDOTHELIAL SYSTEM AND HORMONE REFRACTORINESS. A. S. GORDON, W. KLEINBERG and H. A. CHARIPPER, J. Exper. Med. **70**:333, 1939.

Young splenectomized female rats that were free of latent infection showed greater increases in ovarian weight in response to injections of pregnant mare's serum than young normal rats with spleens intact. The regression in ovarian weight which occurred after about a month in such treated splenectomized animals could be prevented by repeated injections of an agent like trypan blue, which causes blockage of the compensating reticuloendothelial elements. The plasma obtained from these splenectomized and splenectomized, dye-blocked animals possessed less antihormone substance than plasma from similarly treated normal animals. Young female rats heavily infected with Bartonella muris and therefore possessing injured reticuloendothelial systems showed heavier ovaries in response to injections of pregnant mare's serum than did normal, uninfected rats. Similar differences in effect on the testes and seminal vesicles of young normal, splenectomized and bartonella-infected male rats have been obtained using an extract of the urine of pregnant women (follutein). Young splenectomized, and splenectomized, dye-blocked guinea pigs given injections of thyrotropic extract showed heavier and more highly active thyroids than similarly treated normal animals. These results are explained on the basis that the reticuloendothelial system participates in the production of antihormone substances.

FROM AUTHORS' SUMMARY.

HISTAMINE AS THE CHEMICAL MEDIATOR FOR CUTANEOUS PAIN. S. R. ROSENTHAL and D. MINARD, J. Exper. Med. **70**:415, 1939.

Experimental evidence shows that histamine is liberated when the upper layers of the skin are stimulated in the threshold range, although no gross or microscopic evidence of damage to the tissue is demonstrable. A histamine-like substance is recoverable from the anterior chamber of the rabbit's eye on electrical stimulation of the cornea. This substance is liberated in direct proportion to the intensity of the stimulus. Histamine when injected intradermally or applied to the denuded skin (less epidermis and some cutis) or cornea causes pain. That the substance liberated is most likely histamine was shown by its action on the intestinal strip of the guinea pig, which action was not effaced by adding atropine to the bath; by its heat stability; by its neutralization by histaminase and its dialysability through cellophane membranes, and by the fact that thymoethyldiethylamine, which appears to be a specific antagonist to histamine, neutralizes the action of the diffusates of stimulated skin and when injected subcutaneously or rectally abolishes generally the pain responses to pinching, pricking and cutting, and lowers the electrical threshold of the skin markedly without affecting the somatic sensory nerve trunks.

FROM AUTHORS' SUMMARY.

HEMORRHAGIC NECROSIS OF THE ADRENALS IN RATS ON DEFICIENT DIETS. F. S. DAFT and W. H. SEBRELL, Pub. Health Rep. **54**:2247, 1939.

We have observed extensive hemorrhagic necrosis of the adrenal glands of rats on deficient diets. The condition appears to be due most probably to a deficiency in some unidentified dietary factor. These animals have not shown the purpura or the changes in the bone marrow reported by other investigators in rats on diets deficient in various factors of the vitamin B complex.

It appears unlikely that the hemorrhagic adrenal necrosis is part of the syndrome described as panmyelophthisis, although there is not sufficient evidence to prove conclusively that the two conditions are entirely unrelated.

FROM AUTHORS' SUMMARY.

HEMORRHAGIC CORTICAL NECROSIS OF ADRENALS IN RATS ON DEFICIENT DIETS. A. A. NELSON, Pub. Health Rep. **54**:2250, 1939.

The hemorrhagic necrosis of the adrenal cortex and other lesions found in rats on diets deficient in some fraction of the vitamin B complex are described.

The panmyelophthisis which György and co-workers found to occur together with hemorrhagic necrosis of the adrenals in a large proportion of their rats was found in only 1 rat in this series, and this animal had no adrenal lesions.

FROM AUTHOR'S SUMMARY.

POLYPEPTIDE RESPONSIBLE FOR PHENOMENA OF ACUTE INFLAMMATION. E. S. DUTHIE and E. CHAIN, Brit. J. Exper. Path. **20**:417, 1939.

Duthie and Chain have isolated a polypeptide from peptic hydrolysates of blood fibrin, intradermal injections of which in high dilutions caused an immediate increase in the permeability of the vessels of the skin, together with an infiltration of leukocytes. In vitro the substance was positively chemotactic to leukocytes. Polypeptides with similar effects were present in hydrolysates of other proteins digested with pepsin, trypsin and papain. It is believed that these polypeptides are responsible for the changes in permeability of the blood vessels and for the leukocyte infiltration which occur in inflammation.

TOXICITY OF INDENE. G. R. CAMERON and C. R. DONIGER, J. Path. & Bact. **49**: 529, 1939.

Indene exerts toxic effects on rats, mice and guinea pigs, producing necrosis of the liver, sometimes of the cytolytic type (acute yellow atrophy), and less frequently splenic and renal focal necrosis. Other organs are unaffected. No significant change has been found in the blood picture. Young rats are much more susceptible than fully grown animals. Since toxic effects are obtained only after exposure to high concentrations or administration of large amounts, indene cannot be regarded as a highly noxious agent. It is felt, however, that a limit should be set to the indene content of such insecticides as heavy coal tar naphtha.

FROM AUTHORS' SUMMARY.

Pathologic Anatomy

CAPILLARY RUPTURE WITH INTIMAL HEMORRHAGE AS A CAUSE OF PULMONARY THROMBOSIS. J. C. PATERSON, Am. Heart J. **18**:451, 1939.

In 2 cases of thrombosis of the pulmonary artery and its branches the various thrombi were attached to the intima at points where hemorrhages into atheromatous plaques had occurred. It would appear that intimal hemorrhage in the pulmonary artery from the rupture of capillaries derived from the arterial lumen may be a cause of thrombosis.

FROM AUTHOR'S SUMMARY.

SPEED OF HEALING OF MYOCARDIAL INFARCTION. G. K. MALLORY and others, Am. Heart J. **18**:647, 1939.

The age of an infarct can be judged accurately from the histologic picture during the first three weeks. After this the estimation is not very accurate. The speed of healing is in part dependent on the size and position of the infarct and in part on the state of the remaining myocardial circulation. Small infarcts are almost completely healed after five weeks. Large infarcts are almost completely healed after five weeks. Large infarcts are completely healed, or undergo no further discernible change, after two months. Rupture of the heart is common during the first week, may occur during the second week but is rare thereafter. Histologically, much of the necrotic muscle has been replaced by connective tissue by the end of the first fortnight. The healing of infarcts in human hearts is similar in most respects to that of experimental lesions in animals except that the process is slower.

FROM AUTHORS' SUMMARY.

CHRONIC HYPERTROPHIC SPINAL PACHYMENINGITIS. G. WILSON, H. BARTLE JR. and J. S. DEAN, Am. J. M. Sc. **198**:616, 1939.

The pathologic incidence of chronic spinal hypertrophic pachymeningitis far outranks its clinical detection, the latter being of some importance, as the syndrome may simulate cord tumor. Moreover, pachymeningitis itself is not infrequently surgically remediable. A clinical study of 15 cases of chronic hypertrophic spinal pachymeningitis is presented, with a pathologic correlation of 12 of these cases.

The outstanding clinical features of an "ideal case" appear to be: adult age, male sex, syphilis, radicular pain, an ill defined sensory level, ataxia as the most prominent symptom referable to the spinal cord, symptoms indicating involvement of the pyramidal tract, vesical symptoms, intracranial signs, lower motor neuron signs more widespread than at the apparent sensory level, with partial block on manometric study of the spinal fluid and certain features of lipoidal nature, as emphasized by Moniz.

The characteristic pathologic features are: lymphocytic infiltration and fibrous hyperplasia of the dura, which may be adherent to the pia-arachnoid; compression of the spinal roots and secondary degeneration of the cord, particularly of the posterior columns and of the dorsal and ventral spinocerebellar tracts, and chronic circulatory insufficiency with resultant myelomalacia of the gray and white matter over several segments above and below the level of greatest involvement.

FROM AUTHORS' SUMMARY.

COR PULMONALE DUE TO OBSTRUCTION OF THE PULMONARY ARTERY BY SYPHILITIC AORTIC ANEURYSMS. C. F. GARVIN and M. L. SIEGEL, Am. J. M. Sc. **198**:679, 1939.

Heart failure due to syphilitic aortic aneurysm is usually caused by dilatation of the aortic valve ring with resultant aortic insufficiency. Heart failure due to obstruction of the pulmonary artery from pressure of a syphilitic aortic aneurysm is an extraordinary occurrence. Three such cases and similar ones previously reported permit the following conclusions: The aortic aneurysm may be large or small. It bulges anteriorly and to the left, thereby compressing the pulmonary artery. The pulmonary artery is obstructed by (1) simple pressure, (2) by a viselike action or (3) by erosion of the aneurysm into the lumen (without rupture). The heart shows hypertrophy and dilatation of the right side, these changes constituting, in terms of modern terminology, cor pulmonale. Clinically, myocardial insufficiency, especially of the right side of the heart, is evident. The aneurysm may or may not give physical signs of its presence. Roentgen studies show the aneurysm in such a position that it could press on the pulmonary artery, and the electrocardiograms reported show right axis deviation. Under such circumstances, if all other causes of heart failure have been excluded the diagnosis can be considered probable.

FROM AUTHORS' SUMMARY.

ANGINA PECTORIS IN HEREDITARY XANTHOMATOSIS. C. MULLER, Arch. Int. Med. **64**:675, 1939.

Hereditary heart disease due to xanthomatosis is fairly common. It is believed to have been demonstrated as a dominant factor in 17 families. Xanthomatosis gives rise to a form of arteriosclerosis which is etiologically and consequently clinically different from ordinary arteriosclerosis. It is possible that it may be different anatomically, too. Xanthomatous deposits may cause valvular lesions, but far more commonly the changes are in the coronary arteries, with angina pectoris. This may occur in young but occurs more frequently in middle-aged and old persons. Symptomatically this form of angina pectoris does not differ from the usual form. In addition to chronic and long-continued heart disease, the condition may cause sudden death. Infarction of the myocardium is also a frequent result. Hypercholesteremia is present, most marked in connection with xanthoma tuberosum, but there is no definite relation between hypercholesteremia and xanthomatous deposits in the skin. Xanthomatous cardiac lesions probably may develop in persons who have no evidence of xanthomatosis in the skin. Xanthoma tuberosum and xanthelasma may be overlooked in clinical examinations and may be confused with other cutaneous conditions also. The occurrence of heart disease in families should direct attention to xanthomatosis, especially when rheumatic fever, syphilis or hypertension does not appear to play any role. In the cases reported here, hypertension was infrequent. Finally, it seems possible that causal and prophylactic treatment may prove to be of value.

FROM AUTHOR'S SUMMARY.

INCIDENCE OF FATAL CARDIOVASCULAR DISEASE IN CHARLESTON, S. C., WITH PARTICULAR REFERENCE TO HYPERTENSION. T. M. PERRY and S. M. LANGSAM, Arch. Int. Med. **64**:971, 1939.

In a series of 2,066 consecutive autopsies performed in Charleston, S. C., all cases in which death was from cardiovascular disease have been studied, using the clinical record, the autopsy protocol and, in many instances, the microscopic slides. These cases were then classified according to etiologic factors. Hypertensive cardiovascular disease was the etiologic factor in more than half the cases. When only cases of congestive heart failure were studied, it was noted that hypertensive cardiovascular disease again more than equaled all other etiologic types of heart disease combined. The incidence of hypertension was particularly high in the Negro race, and especially in Negro males. While hypertension was common in white persons also, arteriosclerotic disease (without hypertension) was almost as frequently a cause of death. Syphilitic cardiovascular disease was seldom encountered in the white patients in this series.

Each etiologic group of cases of vascular disease was further divided according to the manner of death, and the average age at death was shown. In almost every category the Negroes died earlier of vascular disease than did the white patients. Coronary thrombosis, either with or without hypertension, was seldom encountered in the Negroes, but in the white group it was common. An attempt to classify the cases of hypertensive cardiovascular disease according to the factors bringing about hypertension met with little success. The annual variation in deaths from hypertensive cardiovascular disease in this locality was studied, and the oscillations in the graph were shown to be greater than would be expected if chance alone were the important factor. This offers a lead for consideration in attempts to learn the cause of hypertensive disease. Seasonal variation in the number of deaths from hypertensive diseases was also shown, but this variation could be accounted for by chance error.

FROM AUTHORS' SUMMARY.

CAPILLARY STRUCTURE IN SCHIZOPHRENIA. D. M. OLKON, Arch. Neurol. & Psychiat. **42**:652, 1939.

For a number of years Olkon has studied the cutaneous capillaries in healthy and in schizophrenic persons as to size, shape, color, rate of flow and other fea-

tures. The capillary system in a normal person differs from that in a schizophrenic patient. The number of capillaries in the latter is reduced; their shape is not comma-like, as normally it is, but spiral, crescent, stellate and even tortuous, as in arteriosclerosis; the color is reduced, and the flow of blood is inconstant, either slower or more rapid than in normal persons; capillary hemorrhage occurs, and the lumen is dilated. On the whole, the capillary system in schizophrenia strikes one by its inadequacy and disharmony. According to the author, some association exists between the condition of the capillaries and the mental state, and a derangement of the capillary structure may have some bearing on the severity of the schizophrenic process.

G. B. HASSIN.

GENESIS OF CEREBRAL ABSCESS. F. A. CARMICHAEL JR., J. W. KERNOHAN and A. W. ADSON, Arch. Neurol. & Psychiat. **42**:1028, 1939.

The authors describe what they term the chain of events in the formation of an abscess of the brain. They recognize four stages which may merge into one another. In the first stage focal necrosis of the cerebral tissues is associated with great activity of the microglia, hyperemia, occlusive endarteritis and perivascular infiltration. In the second stage fibroblasts are much in evidence (fibrosis), especially in the region of hyperemia; astrocytes are increased in number, and the microglia cells appear as gitter cells. In the third stage fibroblasts are more numerous, and the process is in general an exaggeration of the phenomena of phase II, but the distinguishing feature is the activity of the astrocytes. In the fourth stage, that of repair, newly formed vessels are seen without perivascular infiltrations; fibrosis is more extensive and intensive. In the advanced stage four layers are recognized—the central necrotic zone, the revascularizing granulomatous border, the zone of hyperemia and fibrosis and the external zone of gliosis. There may be delay or modification of the cell reaction; irregular variations occur as necrotic cerebritis, purulent meningitis and diverticulation (irregular extension of the abscess before "actual delimitation"—membrane or capsule formation—has occurred).

G. B. HASSIN.

MYELOBLASTS, LYMPHOBLASTS AND ACUTE SPLENIC TUMOR CELLS. A. R. RICH, M. M. WINTROBE and M. R. LEWIS, Bull. Johns Hopkins Hosp. **65**:291 and 311, 1939.

The behavior of lymphoblasts from normal lymph nodes and from the blood of patients with lymphoid leukemia has been compared by means of motion pictures with that of myeloblasts from normal bone marrow and from the blood of patients with myeloid leukemia. The comparison has revealed a striking difference in manner of locomotion between lymphoblasts and myeloblasts. These observations provide a new type of evidence against the "unitarian" interpretation of blood formation, which assumes, on the basis of the appearance of the cells in stained preparations, that lymphoblasts and myeloblasts are identical. The manner of locomotion of the mononuclear phagocyte ("monocyte"; "histiocyte") has conformed to that described for this cell by other observers, and in this study it has been found to be entirely different from that of either the lymphoblast or the myeloblast.

Infection is not necessary for the occurrence of acute splenic tumor. This condition represents a reaction to the parenteral presence of foreign protein, whether bacterial or nonbacterial. Lymphoblasts, myeloblasts and histiocytes (monocytes) can be distinguished from each other by their type of locomotion. Motion picture study of the large basophilic mononuclear acute splenic tumor cells in comparison with lymphoblasts, myeloblasts and histiocytes (monocytes) shows that the type of locomotion of the acute splenic tumor cell is precisely like that of the lymphoblast and quite different from that of the myeloblast or the histiocyte (monocyte). It is therefore concluded that the large basophilic mononuclear cells which proliferate in the spleen in acute splenic tumor are lymphoid in character.

Lymph nodes draining infected tissues or a site into which foreign protein has been injected exhibit lymphoid cell proliferation entirely like that characteristic of acute splenic tumor. Since the present studies identify as lymphoid the cells which proliferate so promptly following parenteral injection of foreign protein, it is concluded that one function of the lymphocyte is concerned in some way with the body's reaction to foreign protein. FROM AUTHORS' SUMMARIES.

ARTERIOSCLEROSIS AND ESSENTIAL HYPERTENSION. S. S. BLACKMAN JR., Bull. Johns Hopkins Hosp. **65**:353, 1939.

Cross sections of portions of main renal arteries in a series of 50 cases of essential hypertension disclosed arteriosclerotic plaques projecting into the lumens of the arteries in 43 cases. In most of the involved arteries the plaques were localized in segments of the vessels near the aorta, and they caused partial occlusion varying from moderate constriction to marked stenosis of the vessels. In 2 cases, besides arteriosclerotic lesions, there were old thrombi which almost completely occluded the arteries. One or both of the main renal arteries were stenosed to a marked degree in 27 cases. The difference between the inside and the outside diameter of the narrow arteries in this group varied from 4 to 6 mm. In 5 of these cases both renal arteries were nearly occluded, and in 11 other cases a single main renal artery was almost completely stenosed. The lumens of the very narrow arteries in these 16 cases were reduced to small clefts, measuring 1.5 mm. or less in width. In a second group comprising 16 cases, a moderate degree of stenosis of one or of both of the main renal arteries was found. The difference between the inside and the outside diameter of the moderately narrowed arteries varied from 3 to 3.5 mm. In 7 cases the main renal arteries so far as they could be examined did not appear to be significantly narrowed. There was a difference between the inside and the outside diameter of the arteries in this group which varied from 1 to 2.5 mm. There was clinical and histologic evidence of vascular nephritis in 28 of the cases. Little difference was found, however, in the incidence and degree of stenosis of the main renal arteries in the cases with and the cases without nephritis. In a group of cases in which there had been hypertension and renal insufficiency and in which, therefore, the lesions of acute arteriolar necrosis might occur, it was found that the arteriolar lesions were present in 28 per cent of 14 kidneys whose main renal arteries were very much narrowed (1.5 mm. or less in width). On the contrary, acute arteriolar lesions were found in 87 per cent of 39 kidneys whose main renal arteries were narrowed by more than 1.5 mm. in least diameter. The higher incidence of arteriolar lesions in the latter group of kidneys suggests that the genesis of the lesions may be similar in man and in experimental animals. In all the cases of hypertension in this series the intrarenal arteries were the site of arteriosclerotic changes, and the arteriosclerotic plaques were found to cause a marked degree of stenosis of one or of both of the main renal arteries in over half of the cases. It seems not unlikely that these arterial lesions may have caused sufficient partial occlusion of the renal arteries and their branches to induce chronic hypertension by the mechanism which Goldblatt and others have shown to be effective in experimental animals. A degree of arteriosclerosis and stenosis of main renal arteries comparable to that seen in some of the cases of hypertension was found in 5 control cases. In these 5 control cases the state of the myocardium, of the kidneys or of the renal arteries themselves was such as to appear to offer a reasonable explanation for the absence of hypertension. FROM AUTHOR'S SUMMARY.

MUSCULATURE OF THE LUNG. S. ENGEL and G. H. NEWNS, J. Path. & Bact. **49**:381, 1939.

In childhood there is no muscle at all in the pleura or interstitial tissue of the lung, and in the air spaces there exist only fine fibers and not thick bundles. In

cases of pathologic solidification of the lung the musculature of the air spaces—much more rarely of the lymphatics—becomes slightly hypertrophied and is then more easily seen toward the periphery of the ductules. Thus it is assumed that fine fibers must be present also in the periphery; otherwise hypertrophy would be out of the question. Exceptional cases like the one described in this paper, in which muscular tissue was present in large amount, suggest that there must be an additional factor to which the enormous increase of muscle is due. This possibly is a congenital predisposition. The condition described by Baltisberger was apparently pathologic, perhaps belonging to the same category as that of the 6½ year old girl described here. Baltisberger appears to have been wrong in supposing that the large amount of smooth muscle described by him was a normal constituent of the lung tissue. Cases such as those just mentioned might be the starting point of the so-called muscular cirrhosis of the lung which is described from time to time, the origin of which has been obscure. It is even possible that congenital predisposition to muscular hypertrophy may play a part in the etiology of asthma.

FROM AUTHORS' SUMMARY.

APICAL SCAR DEVELOPMENT. J. DAVISON, J. Path. & Bact. **49**:483, 1939.

The earlier stages of the development of the apical scar were studied in 37 persons under 36 years of age as encountered in 130 consecutive autopsies. The earliest stage, recognizable from the age of 10 years, consists of an accumulation of dust-laden cells in the subpleural alveoli of the apex, especially in those of the pleuroseptal junctions. In the next stages there is proliferation of elastic and collagen fibers in the alveolar walls. Collagenous thickening of the pleura occurs relatively late. Later stages show contraction and obliteration of the alveoli, with diffusion of the dust in phagocytes throughout the interstitial tissue of the scar. Such processes of scar formation are obviously present in both apexes in the great majority of adults, but the lesser degrees in younger subjects are easily overlooked unless several blocks from both apexes are taken. The absence of evidence of active tuberculosis throughout the described sequence of changes except when generalized tuberculosis has been present argues strongly against the tuberculous origin of these lesions, as does also the paucity of evidence of healed tuberculosis in or near the scars.

FROM AUTHOR'S SUMMARY.

ENDOCRINE GLAND IN THE RENAL ARTERIAL WALL. N. GOORMAGHTIGH, Bruxelles-méd. **19**:1541, 1939.

In addition to the fusiform smooth muscle fibers there are in the media of the arteriole globular cells without myofibrils, which Goormaghtigh calls afibrillar cells. They differ in behavior from the smooth muscle fibers under certain experimental conditions. They resemble cytologically certain cells in the arteriovenous anastomoses of the glomus of the skin and in the carotid body. Such cells are found regularly in the walls of the arterioles of the renal cortex where these vessels divide into the capillaries of the glomeruli. In the normal cat and still more distinctly in the rabbit these cells show secretory granules, with evidences of cyclic changes: a homogeneous acidophilic appearance, followed by appearance of acidophilic granules, which later become basophilic. In the normal dog such granules are not found. This report is based on a study of kidneys of dogs and rabbits in which hypertension was produced experimentally. In 12 dogs the method of Goldblatt was employed. Two were observed for periods varying from eight to seventeen months, with systolic blood pressures varying from 182 to 206 mm. of mercury. Ten dogs which were observed for periods varying from twenty-four hours to twenty-five days showed blood pressures varying from 172 to 238 mm. of mercury. The essential microscopic change was found in the afibrillar cells. They were increased in size and number at the vascular poles of the glomeruli, and from there they invaded the glomeruli proper. Occasionally the

encroachment on the arteriolar lumen led to collapse of the glomerulus. Intralobular arterioles were observed to contain afibrillar cells with multiple nuclei. Transformation of muscle cells into afibrillar cells was also seen. Secretory granules and vacuoles were commonly present in the characteristic cells. In 4 rabbits hypertension was produced by the method of Drury. The kidneys of 2 rabbits presented no gross changes but showed pronounced microscopic changes of the type seen in the dogs. These changes were limited to the subcapsular layers. Far more extensive and intensive were the changes in the other 2 rabbits, in which more severe ischemia led to pronounced gross changes. The granular changes in the cytoplasm of the afibrillar cells were not of degenerative nature; the granules are interpreted by Goormaghtigh as secretory granules of the hormone and as responsible for hypertension. While arterioles of the entire body contain these afibrillar cells, the latter do not necessarily have to be all functionally identical. A close special and functional relation was demonstrated between these cells in the arterioles of the kidney and certain segments of the convoluted portions of the kidney. The proliferation of the afibrillar cells modifies the reactivity of the arteriolar walls, thus introducing an additional and, according to some authors, essential factor for the development of hypertension.

I. DAVIDSOHN.

CHANGES IN THE MEDULLA OBLONGATA IN HYPERTONIA. K. E. RUCKERT and H. DEILMANN, Beitr. z. path. Anat. u. z. allg. Path. **102**:443, 1939.

The medulla was investigated in 49 cases of hypertonia. No changes were observed in the substantia reticularis grisea. Of 33 medullas stained with sudan, 6 showed pronounced fatty degeneration and high grade stenosis of the arterioles, whereas the rest revealed little or no fatty change. In sharp contrast, the arteries of medium and large caliber were normal. The state of the basal cerebral arteries was of interest in that in only 10 of the 49 cases was there severe atherosclerosis. In 5 cases circumscribed nodules of fat-containing glial cells were found. These foci apparently had their origin in a local circulatory disturbance. It is concluded that the arteriolosclerosis is not the cause but the effect of hypertonia and that there is no morphologic basis for the theory that hypertonia and hypertension are secondary to changes in the vasomotor center.

R. J. LEBOWICH.

SPONTANEOUS RUPTURE OF AORTA IN TWO BROTHERS. H. VON MEYENBURG, Schweiz. med. Wchnschr. **69**:976, 1939.

Gsell in 1928 described a previously unknown disease of the ascending portion of the aorta, which was designated by Erdheim in 1929 as "medionecrosis idiopathica aortae." It is characterized by focal necrosis of the media, affecting at first the muscle fibers, regeneration of the elastica, appearance of spaces filled with mucoid matter, formation of dense scars and imperfect degeneration of muscle and elastic tissue, leading to transformation of the media. A striking feature is the absence of inflammatory changes. Toxic influences, hypertension and changes in the vasa vasorum have been considered as possible etiologic factors. Meyenburg reports 2 instances of this condition in two brothers. In a 38 year old factory worker who died suddenly after a meal necropsy revealed a horizontal rupture of the ascending part of the aorta and a hemorrhage into the pericardial sac. A 45 year old brother of this patient, a farmer, died suddenly a few months later, a short while after sexual intercourse. The autopsy disclosed two horizontal ruptures of the ascending portion of the aorta with a dissecting aneurysm extending proximally to the origin of the left coronary artery and distally to the level of the diaphragm. The microscopic changes were identical with those described by Gsell and Erdheim. The occurrence of identical lesions in two brothers of similar age forces the consideration of hereditary predisposition as a factor in the pathogenesis.

I. DAVIDSOHN.

Pathologic Chemistry and Physics

SEASONAL VARIATION IN THE WATER CONTENT OF THE RESPIRATORY TRACT. E. M. BOYD and G. M. JOHNSTON, Am. J. M. Sc. **199**:246, 1940.

The percentage of water in the lungs of guinea pigs and albino rats was found to remain remarkably constant. Pearson's coefficient of variation averaged about 1. In the albino rat a significant increase in the water content occurred during the first cold spell of the autumn weeks, and a significant drying of the respiratory tract took place in the winter in animals housed in a building heated by a hot water system. These changes affected all parts of the respiratory tract, especially the trachea and the proximal part of the lung in the autumn and the distal part of the lung in the winter. The correspondence of these seasons to those in which respiratory infection is epidemic has been noted. FROM AUTHORS' SUMMARY.

Microbiology and Parasitology

EPIDEMIC ENCEPHALITIS IN NORTH DAKOTA. P. J. BRESLICH, P. H. ROWE and W. L. LEHMAN, J. A. M. A. **113**:1722, 1939.

Twenty-three instances of acute epidemic encephalitis have occurred in North Dakota. This type of disease had not previously been observed in North Dakota. Clinically it resembled the St. Louis type of acute encephalitis. Pathologically it differed from the St. Louis type in that foci of demyelination occurred in the basal ganglions, pons and medulla oblongata. Serum from only 1 of 6 patients who recovered neutralized the virus of St. Louis encephalitis, while serum from 4 of these 6 patients neutralized the virus of western equine encephalomyelitis. The virus of lymphocytic choriomeningitis was not neutralized by serum from these 6 patients. FROM AUTHORS' SUMMARY.

VIRUS OF INFECTIOUS AVIAN ENCEPHALOMYELITIS. P. K. OLITSKY, J. Exper. Med. **70**:565, 1939.

The transmissible causal agent of infectious avian encephalomyelitis of young chickens is a virus with traits of its own; it is distinct from that of equine encephalomyelitis. The results of experiments provide a basis for the identification of the avian virus. The criteria relate to its antigenicity and serologic reactions, to its size and various other physical properties, to its pathogenicity by various routes of inoculation and to its capacity to induce specific histologic lesions. FROM AUTHOR'S SUMMARY.

EASTERN EQUINE ENCEPHALOMYELITIS. L. S. KING, J. Exper. Med. **71**:95 and 107, 1940.

A fresh strain of the virus of equine encephalomyelitis is infectious for adult mice in high dilutions by all modes of peripheral inoculation. A fixed strain has very limited invasive power when injected peripherally unless it is placed in fairly close contact with nerve cell bodies, as in the intranasal or the intraocular route. For fixed virus the effectiveness of the mode of inoculation may be graded in the following descending order: intracerebral, intraocular and intranasal, intravenous, intraperitoneal, intramuscular, subcutaneous. Fixed virus has a very limited power of invading the central nervous system along the axons of peripheral nerves, even when injected directly into the nerve. Infants are more susceptible to infection than are adults. But even in infants intraperitoneal inoculation with fixed virus is significantly less effective than similar inoculation with fresh virus. Trauma of the brain does not increase the effective titer of fresh or fixed viruses but may shorten the period of incubation for fresh virus. With intramuscular injection of fixed virus, a pronounced facilitating effect may be produced by simultaneous intra-

peritoneal injection of 0.20 to 0.25 cc. of 50 per cent glycerin. Other irritants tried are without effect.

In infant mice affected with equine encephalomyelitis the first pathologic disturbance is an inflammatory reaction; the corresponding reaction in adult animals is usually less pronounced. A characteristic type of parenchymal damage appears to be independent of the inflammation. In such foci of injury there is initially vacuolation of intercellular tissue. Neurons in such areas are at first intact; later they show cytoplasmic changes and finally nuclear alterations. Complete disintegrations of tissue and all its elements may be the end result.

From Author's Summaries.

Analysis of Tubercle Bacillus. H. J. Corper, J. Infect. Dis. **66**:23, 1940.

On a simple synthetic nonprotein medium (Wong-Weinzirl, 500 cc.) maximum growth of human tubercle bacilli occurs within two to three months, while liberation of tuberculoprotein into the medium takes place mainly after maximum growth is attained.

The bacillary body sensitizes primarily to tuberculoallergy and serves to immunize against virulent infection, while the natural filtrate, containing tuberculoprotein, sensitizes to anaphylaxis and provokes anaphylactic shock and allergic intoxication but does not sensitize to allergy nor does it specifically immunize against virulent infection.

Tuberculoanaphylaxis, tuberculoallergy and tuberculoimmunity show distinctive characteristics, which lead to the conclusion that they are separate and apparently unrelated biologic phenomena.

From Author's Summary.

Bacteriostatic Action of Sulfanilamide. A. Fleming, J. Path. & Bact. **50**:69, 1940.

Experiments are described showing the bacteriostatic power of sulfanilamide and sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine; preparation used, M & B 693) in broth, serum and blood, with or without leukocytes, and on solid culture medium. All such experiments show that these substances have a powerful bacteriostatic action on small numbers of Streptococcus pyogenes but that the action on massive implants is negligible. Sulfanilamide injected intravenously into a rabbit immediately confers on the blood increased bacteriostatic power. The significance of this is discussed. Thick suspensions of bacteria in combination with sulfanilamide or sulfapyridine give off substances which inhibit the bacteriostatic action of the chemical, but on dilution the bacteriostatic power returns. Peptone added to sulfanilamide has a similar effect, and the degree of inhibition of the bacteriostasis appears to depend on the concentration of the peptone and to be independent (within limits) of the concentration of sulfanilamide. Dead streptococci added to human blood favor the growth of small implants of the same streptococci.

From Author's Summary.

Immunology

Antibodies to Spirochetes in Syphilitic Serum. H. Eagle and R. B. Hogan, J. Exper. Med. **71**:215, 1940.

Since suspensions of cultured spirochetes contain antigenic factors which react specifically with syphilitic serum, some of which are not present in ordinary Wassermann and flocculation "antigens," they may prove even more valuable than these tissue extracts in the serodiagnosis of syphilis.

From Authors' Summary.

HOMOLOGOUS SERUM PROTEINS IN TISSUE CULTURES. K. LANDSTEINER and R. C. PARKER, J. Exper. Med. **71**:231, 1940.

Connective tissue cells (fibroblasts) derived from skeletal muscle of 12 day old chick embryos were cultivated for almost eight months (thirty-five weekly passages) in rabbit plasma and rabbit embryo tissue juice diluted with Tyrode's solution. When fluids separated from these cultures were tested with immune precipitins developed against chicken serum, they gave positive reactions which showed no tendency to diminish with an increasing number of culture generations. Barring the intervention of unknown precipitable substances, these results indicate that connective tissue can produce proteins which are identical with, or closely related to, serum proteins. The experiments further demonstrated that tissues cultivated in a foreign plasma do not lose their species specificity.

FROM AUTHORS' SUMMARY.

SENSITIZATION WITH SIMPLE CHEMICAL COMPOUNDS. K. LANDSTEINER and M. W. CHASE, J. Exper. Med. **71**:237, 1940.

A method has been described by which sensitization to a simple chemical, picryl chloride (2,4,6-trinitrochlorobenzene) can be satisfactorily attained by means of intraperitoneal injections of the compound when killed tubercle bacilli suspended in liquid petrolatum are used as an adjuvant. Sensitivity of the contact dermatitis type results therefrom. It follows that although cutaneous sensitization of this type is most easily obtained by dermal application, this route of administration is not a necessary condition for such sensitivity. FROM AUTHORS' SUMMARY.

PULMONARY REACTIONS IN ANAPHYLAXIS. A. GARIPUY, Ann. Inst. Pasteur **63**:190, 1939.

Persistent pulmonary reactions were produced in rabbits sensitized to horse serum when repeated shocking doses were administered. These lesions persisted for at least fifteen days, whereas a single shock dose produced only transitory changes. Inoculation by the intratracheal or the intravenous route provoked the most constant reactions. Subcutaneous injection occasionally resulted in pulmonary lesions, but intraperitoneal inoculation failed to produce such changes.

The interstitial mesenchymatous and vascular lesions showed necrotic, exudative and proliferative changes similar to those observed in the Arthus phenomenon and in some instances resembled progressive pulmonary sclerosis. In the altered areas of the lung the bronchial epithelium remained intact, but the alveolar cavities, arteries and especially the veins were sometimes obstructed by an acidophilic hyaline fibroid substance. Characteristic was a leukocytic infiltration of the peribronchial connective tissue, alveolar cavities and alveolar walls. The alveolar cavities also contained red cells and macrophages with pigment. The elastic tissue was markedly altered.

These pulmonary lesions were partly due to the effect of the last injection and partly to the cumulative effect of four to ten previous shocks.

J. B. GUNNISON.

Tumors

HISTOLOGIC ASPECTS OF DIBENZANTHRACENE MOUSE SARCOMA. W. H. LEWIS, Am. J. Cancer **37**:521, 1939.

The sections of 22 of our first 50 primary dibenzanthracene mouse tumors were found to contain modified skeletal muscle fibers, muscle giant cells, myoblasts, spindle cells and invaded host tissues. Sections of the remaining 28 consisted principally of spindle cells without modified muscle. There are similarities and differences in the primary and later generations of the latter, but the histologic

sections do not enable one to determine whether the differences are due to permanent differences of the cells or to temporary differences imposed by the host environment. With rare exceptions, all the modified muscle elements disappear from the later generations, and sections of the latter are similar to those of the spindle cell sarcomas without modified muscle. The disappearance of the modified muscle elements in later generations suggests that they either dedifferentiate into spindle cells, which are malignant and indistinguishable from those of connective tissue origin, or are lost or die out. We cannot determine from the histologic sections either the origin of the malignant spindle cells or how much or how little they differ from one another.

FROM AUTHOR'S SUMMARY.

EFFECT OF ZINC ON MARSH-BUFFALO ADENOCARCINOMA. F. BISCHOFF and M. L. LONG, Am. J. Cancer **37**:531, 1939.

In an experiment on 50 virgin mice of the Marsh-Buffalo strain the injection at the age of 2 to 3 months of two series of 0.05 mg. doses of zinc sulfate in the area of the mammary glands significantly delayed the appearance of mammary adenocarcinoma. In the fifteenth month of age 73 per cent of the controls, as compared with 38 per cent of the zinc-dosed mice, had tumors. In another experiment, on 44 pairs of litter mates of the same strain, the injection was begun before the onset of sexual maturity. Similar results were obtained. It is concluded that zinc does not exert a synergistic effect in the genesis of the Marsh-Buffalo tumor but destroys or holds in abeyance the development of the cancer-susceptible cells.

FROM AUTHORS' SUMMARY.

EXTRACHROMOSOMAL INFLUENCE ON THE INCIDENCE OF MAMMARY TUMORS IN MICE. W. S. MURRAY and C. C. LITTLE, Am. J. Cancer **37**:536, 1939.

Some extrachromosomal influence, which is ten times as powerful as any possible chromosomal factor, is instrumental in determining whether or not mammary cancer appears in the first outcross generations. This influence becomes noneffective after eight generations of back crossing. Concentration of the chromatin of the high mammary cancer strain does not return the incidence of cancer to that obtained in the first hybrid generation or to that in the original cancer strain. The tendency to have mammary cancer is not mendelian in nature.

FROM AUTHORS' SUMMARY.

CARCINOMA OF THE LUNG. R. D'AUNOY, B. PEARSON and B. HALPERT, Am. J. Path. **15**:567, 1939.

Seventy-four cases of primary carcinoma of the lung were encountered in 6,623 autopsies on persons over 1 year of age. Males and females were represented in the proportion of 11:1. The age range was from 21 to 75 years. The average duration of illness was five months. Thirteen patients died in the fifth, 33 in the sixth and 19 in the seventh decade of life. In almost half of the cases the primary growth was located in one bronchus or the other. In 37 of the 74 cases the growth was squamous cell; in 21 it was reserve cell, and in 16 it was columnar cell carcinoma.

FROM AUTHORS' SUMMARY.

ROUS I TUMOR-PRODUCING AGENT. C. R. AMIES and J. G. CARR, J. Path. & Bact. **49**:497, 1939.

Methods are described for the preparation of highly active concentrated suspensions of the filtrable agent of fowl tumor. The procedure consists essentially in sedimenting the agent from cell-free tumor extracts by centrifugation at p_H 5-5.5 and digesting the resuspended deposit with commercial trypsin at p_H 9. The agent is recovered by further fractional contrifugation. By this process a considerable

degree of purification is also effected, but it has not yet been possible to obtain completely homogeneous suspensions of the tumor agent. The inhibition of the Rous I and des Ligneris sarcoma agents by rabbit antifowl serum appears to depend on a specific antigen-antibody reaction. The serum of rabbits which have been repeatedly inoculated with large doses of tumor agent suspension contains neutralizing antibodies for the agent and also antifowl hemolysins and precipitins. The latter are present only in relatively low concentration and may have been produced in response to impurities (cell débris) present in the suspensions used for inoculation. Absorption of rabbit antifowl serum or rabbit serum containing antibody for the tumor agent with normal chick embryo tissue completely removes the inhibitory properties for the tumor agent. Fowl serum containing antibody for the tumor agent, on the contrary, is not affected by absorption with chick embryo. Isoantibodies play no part in the inhibition of the tumor agent by Rous tumor–immune fowl serum. These experiments appear to support the belief that the tumor agent has at least two antigenic components, corresponding to two antibodies, one of which is present in Rous tumor–immune fowl serum and the other in rabbit antifowl serum. The findings are therefore in agreement with those reported by Gye and Purdy, but the present authors consider that both these antigenic factors are intrinsic to the agent, whereas the former workers believed that the "fowl" factor was extrinsic.

FROM AUTHORS' SUMMARY.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

S. A. Levinson, *President*

Edwin F. Hirsch, *Secretary*

Regular Monthly Meeting, April 8, 1940

Influence of Differential Stasis Within the Gallbladder on the Composition of Gallstones. Dallas B. Phemister.

Gallstones formed in the gallbladder in the presence of a considerable degree of obstruction of the cystic duct and of mild inflammation usually contain calcium salts as well as cholesterol and are dark in color. Aronson has shown that the dark color is due to the presence not only of bile pigments but also of a dark material which does not give the reactions for bile pigments but which appears to be a pyrrole ring derivative of the pigments.

When a number of ordinary cholesterol pigment stones grow to a size at which they partition the gallbladder, stagnation of the bile results in the compartments thus created. The stagnation increases in degree, proceeding from the cystic duct to the fundus, and any further growth of the gallstones is of material which has increasing amounts of calcium salts and of dark coloring matter, proceeding from the end of the cystic duct to the fundus. Four typical cases in which this differential composition was observed are presented.

A Parasite Resembling Encephalitozoon Found in White Rats. F. B. Gordon.

The parasites known as Toxoplasma and Encephalitozoon, probably protozoal, have been described as occurring naturally in a number of different animal species. Interest in them has increased recently because several cases of human infection with this type of parasite have been encountered. There have been published a report of Toxoplasma infection of the lung of a rat and another of infection induced in a rat with Encephalitozoon from a rabbit. Apparently there has been no previous report of these parasites being observed in the central nervous system of the normal rat.

The olfactory bulbs of 15 albino rats, bred at the National Institute for Medical Research, London, England, were examined histologically. The remainder of the brain and the other tissues, with the exception of the nasal mucosa in some cases, were not examined. In 2 of these rats parasites resembling those described as Encephalitozoon were seen. These 2 rats were healthy and appeared entirely normal. One was uninoculated; the other had received an emulsion of normal mouse brain intranasally twenty-four hours before being killed. The parasites were confined almost entirely to the lamina gelatinosa of the olfactory bulb and occurred in circumscribed masses of varying size, with or without adjacent inflammatory reaction. In the few sites where individual parasites could be seen, they appeared as oval or ellipsoid bodies, sometimes slightly crescentic, approximately 2 microns in length and 1 micron in breadth. Several stains were used, but hematoxylin-eosin appeared to be as satisfactory as any. With this stain a more deeply tinted region could be made out within the cell, but the differentiation was not sufficient to justify a statement that chromatin was present. Mann's stain, containing orange G, gave a bright orange color to the parasites. The inflammatory reaction when present formed a miliary granuloma, consisting of an accumulation mainly of epithelioid cells. Pyknosis and other evidences of necrosis were sometimes

observed; polymorphonuclear leukocytes were not seen. Neither parasites nor lesions were noted in sections of nasal mucosa of these or other rats of the group. Their size and tinctorial qualities place these parasites in the group which has been termed Encephalitozoon.

DISCUSSION

ARTHUR WEIL: Among the rats of our own laboratory I have never observed sporadic encephalitis due to Encephalitozoon. However, among a series of rat brains from B. Zondek's stock at Jerusalem, Palestine, I observed one with this infection. Sections of the brain presented the histologic picture of encephalitis, with perivascular round cell infiltration and focal proliferation of glia. Within the inflammatory foci and within pseudocysts there were numerous oval or round bodies, approximately 2 microns by 1 micron. With the Giemsa stain, they contained a dark blue–staining substance, which in the oval bodies was accumulated at both poles and in the round bodies appeared in a crescent shape, surrounding a reddish center. Among the stock of rabbits of the State Hospital at Elgin, Ill., which were used in different experiments, more than 50 per cent had infections with Encephalitozoon (Toxoplasma?). The oval protozoan-like bodies were in the necrotic centers of the granulomas.

The subject of protozoan encephalitis in man is unexplored. It is possible that isolated cases have been overlooked in the material of many hospitals. The resemblance of the inflammatory reaction to that of epidemic encephalitis may have led to this diagnosis in isolated sporadic cases of infection with Encephalitozoon. Wolfe and Cowen diagnosed Encephalitozoon encephalitis in a case which previously had been published by Dr. R. Richter, of Chicago, as a case of meningoencephalitis neonatorum. They have published 4 cases of encephalitis in infants in which they demonstrated pseudocysts and isolated Toxoplasma (*Am. J. Path.* **15**:657, 1939).

Bacteriogenic Agglutinins and Agglutinogens. I. DAVIDSOHN and B. TOHARSKY.

A bacterium belonging to the genus Corynebacterium was isolated which produces in the test tube, in the serum and in the plasma of man and of some animals, a hitherto unnoted hemagglutinin. This factor reacts with red blood cells of all human groups and with red cells of some animals. The newly isolated bacterium was designated as Corynebacterium H. It is proposed to call the new hemagglutinin—bacteriogenic hemagglutinin. In plasma the bacteriogenic hemagglutinin was produced quicker and more frequently, and its titer was higher, than in serum. Filtrates of cultures of Corynebacterium H produced in serum and plasma the bacteriogenic hemagglutinin, just as the bacterium itself did. The properties of the newly isolated agglutinin are described. Corynebacterium H and its filtrates make red blood cells of man and some animals panagglutinable, thus producing the so-called Thomsen panagglutination phenomenon. By means of absorption experiments it has been shown that the three hemagglutinins in human serum—the isoagglutinins, the normally present nonspecific hemagglutinins which react with the bacteriogenic agglutinogen, and the hitherto unknown bacteriogenic hemagglutinin—are independent of each other, and each can be removed separately. A consideration of the possible significance of the bacteriogenic hemagglutinin as a source of errors in blood grouping must take into account two facts: (*a*) Storing serum in the ice box does not prevent the development of the bacteriogenic hemagglutinin; (*b*) completing the test at 37 C. does not always prevent the occurrence of the agglutination reaction.

DISCUSSION

P. CANNON: Are there any studies of a type in which mass cultures of bacteria would produce a mucoid substance that would be adsorbed and cause this phenomenon?

I. DAVIDSOHN: These bacteria are highly proteolytic in cultures. Filtrates in 10 to 35 per cent concentrations do the same as the organisms.

Experimental Bacillary Dysentery and Its Spontaneous Development in Monkeys on a Diet Deficient in "Vitamin M." MARTHA JANOTA.

Adult monkeys of the species Macaca mulatta with ileal, duodenal and cecal fistulas were fed huge numbers of dysentery bacilli (Flexner and Shiga) which were known to be pathogenic to man. Although cultures of ileal, duodenal and cecal tissues and of feces gave growths of the dysentery organisms from four hours to as long as seven days later, the animals appeared healthy, and no clinical dysentery developed. Dysentery did not develop in kittens fed cultures of Shiga and Flexner dysentery bacilli even after these animals had had their resistance lowered by their being placed in an incubator at 38 C. for many days.

Three groups of monkeys were maintained on a diet deficient in certain components of the vitamin B complex designated as vitamin M by Langston, Darby, Shukers and Day. As previously reported by them, in these monkeys a fulminating blood disease developed, characterized by leukopenia, gingivitis, anemia and diarrhea. Blood counts were made, and feces were taken weekly for culture on sodium desoxycholate–citrate medium. The second group of monkeys was examined by proctoscope at weekly intervals. Spontaneous bacillary dysentery developed in 13 of 29 monkeys on the vitamin M–deficient diet, and Bacterium dysenteriae (Flexner X) was isolated from the feces of these 13 monkeys many times before death and from lesions of their colons at necropsy. The appearance of the organisms in the stools was usually associated with avitaminosis, characterized by leukopenia, gingivitis, nutritional edema or diarrhea. Ulceration of the mucosa of the colon was not observed on proctoscopic examination until the dysentery organisms were found as the predominating flora on sodium desoxycholate–citrate agar.

Six dietary control monkeys maintained on the vitamin M–deficient diet supplemented by brewers' yeast to insure an adequate intake of vitamin M did not have dysentery. This would indicate that the spontaneous dysentery in the monkeys on the vitamin M–deficient diet occurred as a result of avitaminosis and was not due to contact infection. Of the 16 animals on the experimental diet in which spontaneous dysentery did not develop, 3 had been immunized against an animal strain of Flexner dysentery bacilli. Of these 3 monkeys, 2 died as a result of avitaminosis, and Bact. dysenteriae was never isolated and there were no colon lesions at necropsy. The third immunized animal died of pneumonia. Of the animals in which dysentery did not develop, 5 died of pneumonia, 1 of tuberculosis, 6 as a result of avitaminosis and unknown causes, and 1 from experimental dysentery. In those animals in which dysentery developed, a significant increase in agglutinins for Bact. dysenteriae (Flexner) was observed, while control animals had no increase in agglutinins.

These experiments suggest that Bact. dysenteriae (Flexner) may have a commensal existence, being present in the intestines in numbers which escape detection even when an excellent selective medium is used. Monkeys kept in the laboratory on the stock diet plus cod liver oil do not acquire bacillary dysentery. When the resistance of these animals is lowered as has been shown, by placing them on a diet deficient in vitamin M, many of them present spontaneous dysentery. If the carrier situation in man is similar to what it is in the monkey, the high incidence of the disease may be explained.

DISCUSSION

P. CANNON: Studies of this kind indicate the importance of diet. The vitamin deficiency had no effect on the production of antibodies in the blood. Apparently, the depression of resistance is a local condition of tissue and is not general.

S. A. LEVINSON, *President*

EDWIN F. HIRSCH, *Secretary*

Regular Monthly Meeting, May 13, 1940

Studies on the Mechanism of Certain Types of Hypersensitiveness. PAUL R. CANNON and CHARLES E. MARSHALL.

These studies are directed at the problem of the possible relationship of precipitins to some of the allergic phenomena encountered in human beings. Serums from patients allergic to egg protein or to insulin and from patients with tuberculosis have been tested against collodion particles to which either crystalline egg albumin, crystalline insulin or purified tuberculoprotein had been adsorbed. The studies indicate that in a considerable number of such serums precipitins specific for the protein concerned in the particular allergy are demonstrable. These same serums, however, cause no effect on collodion particles bearing adsorbed proteins to which the patients are not hypersensitive. Of particular interest is the demonstration of antibodies specific for various crystalline insulins in diabetic patients hypersensitive or resistant to insulin. This suggests, therefore, that one cause of resistance to insulin may be the local union of insulin and its specific precipitin within the tissues and a correspondingly slower absorption of the insulin. Another interesting feature of the experiments is that these precipitins are present in relatively low concentration, suggesting thereby that hyposensitization may not be impossible to attain.

DISCUSSION

I. DAVIDSOHN: How reactive is the serum of patients after doses of insulin have been given? Have you observed positive serum tests without skin reactions? A woman 60 years of age had received many injections of insulin without reaction. Following an injection of protamine insulin, she had a severe shock, then recovered slowly but not entirely. After several months, following another dose of protamine insulin, she died. There were marked eosinophilic infiltrations of the myocardium.

S. ROSENTHAL: Were complement fixation tests made with the patient's serum? What is the comparative sensitivity of the ring precipitin test in relation to the colloidal suspension? Were you able to get skin reactions without precipitin reactions of the serum?

I. PILOT: Have you tested the precipitin content in passive immunity?

H. C. SWEANY: These tests seem to offer hope in determining active tuberculosis, demonstrating an activity when other tests do not.

PAUL R. CANNON: Any of the serum test tube reactions are not as delicate as the skin tests. The colloidal suspension and the complement fixation test are about equal in degree of sensitivity. This method has been used in cases in which tuberculosis was suspected, and some think that it is valuable in the presence of early tuberculosis, even before the skin test is positive.

Adenoma of the Parathyroid Gland with Hypercalcemia and Bilateral Nephrolithiasis. VINCENT J. O'CONNOR and J. J. KEARNS.

A woman aged 43 years entered Washington Boulevard Hospital for the treatment of bilateral nephrolithiasis. The examinations of the blood demonstrated 4,640,000 erythrocytes, 11,700 leukocytes, 54 mg. of nonprotein nitrogen per hundred cubic centimeters, 3.7 mg. of creatinine, 12 mg. of calcium and 4.3 mg. of phosphorus. The clinical diagnosis was parathyroid adenoma, hypercalcemia and bilateral nephrolithiasis. The left renal pelvis was drained surgically, and several concretions were removed. Death followed ten days after admission to the hospital and four days after the operation. Post mortem there was demonstrated an

enlarged parathyroid (20 by 13 by 10 mm.) at the lower pole of the left lobe of the thyroid. On surfaces made by cutting, it contained a circumscribed adenoma 10 mm. in diameter. Other parathyroid glands were not found. The symmetric thyroid weighed 9.2 Gm. Both kidneys weighed 300 Gm. Their calices and pelves were markedly dilated and filled with multiple concretions and a putrid exudate. The renal tissues had marked pyonephritis.

DISCUSSION

VINCENT J. O'CONNOR: In my experience the association of renal concretions with hyperparathyroid function is much less frequent than others have reported. There was no roentgen evidence of osteitis fibrosa in this patient. She seemed hopelessly sick, and the operation was undertaken on the better kidney.

Atherosclerosis: II. The Lipids of the Serum and Tissues in Experimental Atherosclerosis in Rabbits. SIDNEY WEINHOUSE and EDWIN F. HIRSCH.

The report will be published in full in the ARCHIVES OF PATHOLOGY.

DISCUSSION

S. A. LEVINSON: Several years ago I fed rabbits large quantities of cholesterol, and although the lipid content of the blood was high, there were no deposits in the aorta.

PAUL R. CANNON: Is there an explanation for the gradual decline of the lipids in the blood after the initial rise?

EDWIN F. HIRSCH: Many other phases of this work need investigation. It seems that the fixed tissue phagocytes are important in these feeding experiments, at least in removing particulate lipid material from the blood. Emulsified cholesterol oleate injected intravenously is rapidly removed and is found in the phagocytes of the liver, spleen and other viscera. Apparently, the cholesterol material, whether injected, finely dispersed, into the blood or entering the blood in dispersion after absorption from the intestinal tract, enters the tissue phagocytes.

SIDNEY WEINHOUSE: There is no clear explanation for the failure to produce experimental atherosclerosis, except possibly in some dietary factor. Thyroid extracts and potassium iodide are reported as able to prevent the arterial deposits. The decline in the lipid content of the blood seems to be due to impaired absorption of cholesterol in the intestinal tract. There are marked lipid deposits in the lining and wall of the stomach and small bowel.

Eosinophilic Granuloma of Bone. C. HOWARD HATCHER.

The term "eosinophilic granuloma" describes the essential pathologic characteristics of a peculiar solitary skeletal lesion encountered in 3 children. In 2 of these a long bone was involved. The roentgenographic appearance of central rarefaction, cortical erosion and marked periosteal new bone formation led to a preoperative diagnosis of probable sarcoma. In the third patient, the destructive lesion in the frontal bone appeared as an osteomyelitic focus. Histologic examination was necessary to establish the diagnosis in the 3 patients. At operation, friable granulation tissues without evidence of suppuration were found. Cultures for pyogenic organisms and fungi were sterile. Inoculations into animals gave negative results. The blood serums had no agglutinins for Brucella. The cholesterol content and number of eosinophil leukocytes of the blood were unchanged. The Wassermann reaction of each serum was negative. The tissues removed from the medullary region had a fibroblastic stroma with many macrophages and multinucleated cells and foci of necrosis. Most striking were the focal collections of eosinophil polymorphonuclear leukocytes in the granulation tissues. Curettement of the lesion resulted in prompt relief of pain and rapid healing of the bone. There has been no evidence of recurrence locally or elsewhere in the skeleton.

DISCUSSION

D. B. PHEMISTER: The cause of this condition in the bone is unknown. It seems to be some form of osteomyelitis.

G. KERNWEIN: I observed a similar lesion in the upper part of the femur. The patient complained of severe pain although the examinations disclosed nothing unusual. A roengten film demonstrated changes in bone. Curettage relieved the disturbance.

V. LEVINE: These lesions heal following curettage. The eosinophil leukocytes of the blood are unchanged, as a rule.

NEW YORK PATHOLOGICAL SOCIETY

MAURICE N. RICHTER, *President*

D. MURRAY ANGEVINE, *Secretary*

Regular Monthly Meeting, March 28, 1940

Hemangioendothelioma of Lung: Report of Two Cases. ALFRED PLAUT.

This paper was published in the ARCHIVES OF PATHOLOGY (**29**:517, 1940).

DISCUSSION

MAURICE N. RICHTER: The tumor nodules were all very small, and they were all intravascular. May there not be a relationship to thrombosis in this case? There is a striking similarity between the nodules described and organized thrombi.

ALFRED PLAUT: The absence of thrombosis in the face of such widespread dilatation of the vessels and so much intravascular change was remarkable. There was not the slightest evidence of thrombosis, old or recent, throughout the whole lung, so that one might look around for some mysterious anticoagulating factor. There was no iron pigment or granulation tissue anywhere.

MAUDE E. ABBOTT: I noticed there was atheroma of one of the main branches of the pulmonary artery. Were the main branches much dilated?

ALFRED PLAUT: No.

MAUDE E. ABBOTT: Did the atheroma in the main branch extend into the arterioles?

ALFRED PLAUT: No.

MAUDE E. ABBOTT: I suppose the atheroma was secondary to the large interventricular septal defect. It was interesting that there was a cerebral abscess in this case. In the cases of this type which I have seen there was no stenosis of the pulmonary artery, but an uncomplicated interventricular septal defect with dextroposition of the aorta.

ALFRED PLAUT: The septal defect was found accidentally; the patient had lived without discomfort. She had known, however, since childhood that she had heart disease, and she was forbidden to have children for that reason.

Cor Pulmonale with Bilateral Pulmonary Arterial Aneurysm, Patent Interventricular Septum and Patent Ductus Arteriosus in a Patient Exhibiting Terminally Ayerza's Syndrome. M. W. JOHANNSEN.

A 44 year old white woman was admitted to Bellevue Hospital, Jan. 9, 1940, because of severe hemoptysis. Her past history was singularly uneventful with regard to any etiologic factor for cardiac or pulmonary disease. There was

intense cyanosis of the entire body, with dyspnea and orthopnea. These symptoms appeared for the first time four months prior to admission.

The heart was enlarged to the left and right. A loud systolic murmur was audible. The blood pressure was 160 systolic and 108 diastolic. The liver was enlarged. Marked sacral and dependent edema was noted. The red blood cells numbered 7,140,000; the hemoglobin was 154 per cent. The venous pressure was 28 cm. of water. The determination of arterial oxygen showed only 30 volumes per cent saturation; it rose to 62 per cent after the patient had been in an oxygen tent for several hours. In spite of supportive treatment the patient died eleven days after admission.

Necropsy revealed pleural effusion on the left, pericardial effusion and minimal ascites. In addition, all the organs showed marked chronic passive congestion.

The heart was markedly enlarged with definite cor pulmonale. There was a small defect of the interventricular septum. The foramen ovale was closed. A widely patent ductus arteriosus was noted and the extrapulmonary portions of both pulmonary arteries showed aneurysmal dilatation with sclerosis and calcification, as well as a lamellated blood clot diminishing the lumen. The sclerosis extended well into the smaller branches. A few of the smaller branches of the pulmonary artery were occluded by organized thrombi or emboli. The left side of the heart showed slight dilatation and hypertrophy.

The aorta was diminished in caliber. There was no evidence of syphilis.

We believe that until the occurrence of her symptoms the patient had a compensated adjustment to both arterial-venous shunts which produced pulmonary hypertension, necessitating increased work of the right ventricle. This hypertension may have been responsible in part for the arteriosclerosis. The development of thrombi in the aneurysmal branches gradually obstructed the vessels and contributed to failure of the right side of the heart with increased venous pressure, polycythemia, hemoconcentration and progressive anoxia. It is probable that the venous blood then flowed through the ductus Botalli and possibly through the interventricular septal defect, which would explain the unusual cyanosis and the inability to obtain high oxygen saturation within the arterial blood even under optimal external conditions.

DISCUSSION

MAUDE E. ABBOTT: I do not know much about the histologic study of the pulmonary circulation. That is a field which demands investigation, for there is certainly plenty to be done in it. I have experience of only 1 case which resembled somewhat that presented by Dr. Johannsen, but unfortunately the lungs were not histologically examined. It was a case described under the title of complete congenital heart block in transposition of the great trunks, with a defective interventricular septum, and I described it in my "Atlas of Congenital Cardiac Diseases" (New York, American Heart Association, 1936) and referred to it also in my monograph in G. Blumer's "Bedside Diagnosis" (Philadelphia, W. B. Saunders Company, 1928). It was a case of very complex cardiac anomalies. There was malposition of the left auricle, which lay behind the right; also, cor triloculare biventriculare with transposition of the great trunks, a dilated pulmonary artery and stenosis of the conus of the right ventricle, which gave off the hypoplastic aorta, with diminution in the number of cusps of the tricuspid valve and an accessory cusp in the left auriculoventricular valve; in the latter was a double mitral orifice that carried the blood above the defective interventricular septum through a bulbar septal defect into the aorta. In addition to this, the endocardium of the left auricle was very thick, pulmonary veins were thick walled, and there was a large sacculus behind the entrance of one of these, the size of a walnut. Section of the lungs showed macroscopically smooth-walled cavities, which suggested the appearance of an arteriovenous aneurysm, as did the radicles of the bronchial arteries. The patient was under the charge of Dr. C. F. Moffatt, and was a young man of 20, with marked cyanosis and clubbing of the fingers. He died from an enormous pulmonary hemorrhage. We concluded that he had a congenital arteriovenous pulmonary aneurysm, but, unfortunately, before the

lungs could be examined histologically other than in the routine way they were thrown out, so that the case was not completely studied. However, although there was undoubtedly aneurysmal dilatation there was no thrombosis of the cavities so far as I could see.

M. W. JOHANNSEN: In this case the smaller arteries in the lung show merely marked arteriosclerosis with occasional occlusion by an organizing blood clot.

Life Cycle of Graafian Follicle-Like Tumors. HERBERT F. TRAUT (by invitation).

Furth and Butterworth, Traut and Butterworth and more recently Geist, all working with experimentally produced granulosa cell tumor of the mouse's ovary, have shown that in this animal the tumor does not arise from the celomic epithelium, as has been taught formerly, but forms from elements of the connective tissue of the ovary. This evidence reenforces the thesis of Fischel and his school, who have maintained that the cellular elements of the graafian follicle are developed by differentiation of the mesodermic elements of the ovary. Meyer's hypothesis that differentiated mesoderm is produced in excess of that necessary to envelop the primordial ova and that hence nests of this cellular material remain in the ovary, occasionally for long periods of time, with subsequent development into a neoplasm, is still valid. The cell nests have been demonstrated, and tumors of all dimensions are described in the literature. It is safe to say that no one today believes that the graafian follicle tumors originate from the differeniated follicle, the corpus luteum or even the atretic follicle.

The stimulus which may cause the residual islands of embryonic cells to develop is not known; however, the greatest incidence of the tumors coincides with the period of greatest gonadotropic activity of the anterior lobe of the hypophysis, i. e., during the reproductive period and the early years of the menopause. It may not require too great a stretch of imagination to suppose that the stimulus which causes the normal cells of the graafian system to proliferate has a similar effect on the residual embryonic cell nests, causing them to assume neoplastic tendencies.

Whatever the growth stimulus may be, it is quite clear that it is not always present in a uniform degree. The evidence for this conclusion lies in the fact that the same tumor frequently shows wide variation in the age of different portions. The result of periodic growth is that the neoplasm is frequently an aggregate of groups of cell masses produced at different times and having different degrees of maturity and senescence. The immature cells probably do not secrete hormone, and as the same is true of the senescent cells, it is evident that the composite effect of the tumor in the hormonal sense at any given time must represent the resultant activity of the groups of cell masses which have recently reached maturity. Therefore, the tumor cannot maintain an even level of hormone production but must present wide fluctuations, dependent on the state of growth and nutrition, with the result that the response in other organs must also fluctuate in an irregularly periodic manner. In addition, various collateral events tending to minimize the hormonal effectiveness of the tumor may occur, such as ischemic necrosis and hematoma formation. The latter is very common in the folliculoid type of tumor. These facts explain quite satisfactorily why some women are amenorrheic at the time they come to the physician.

Most graafian follicle tumors produce symptoms which bring the patient to the physician, with the result that the great majority of such tumors are removed before they have reached the end of their life cycle. It is therefore necessary to postulate what some of the terminal aspects of the neoplasms may be. However, it is known that if the tumor is not affected adversely by such accidents as ischemia and hematoma formation the cells tend to mature and to undergo quite typical degenerative changes. The theca cells reach the end of their life cycle sooner than do the granulosa cells. Luteinization occurs in both types of cells, and this is followed by collagenous degeneration, which is replaced by fibrosis. The greater the content of theca cells, the more likely the tumor is to produce

fibrous connective tissue. The granulosa elements, however, being poorly supplied with a supporting framework on which to dispose themselves and being less intimately supplied with blood vessels, are more prone to premature accidents which interrupt the other forms of degeneration that might otherwise ensue.

DISCUSSION

AMOUR F. LIBER: From the classic books of histology and pathology one obtains the impression that there is a very clearcut differentiation between theca cells and granulosa cells and between the types of luteinization which they respectively undergo; in particular, it is stated, first, that the theca cells contain lipids which stain in part at least with sudan III or scarlet red after ordinary fixation in solution of formaldehyde U. S. P., without being mordanted with osmic acid or other substances; second, that the theca cells contain lipids which are in part at least doubly refringent and that they are predominantly cholesterol and cholesterol esters, while, on the other hand, the granulosa cells in the early stages contain no lipids which are microscopically visible. Of course, lipids may be mixed with the cytoplasm in a nonstainable form. Then, when the granulosa cell undergoes luteinization, the lipid becomes apparently phospholipids, which do not stain with ordinary fat stains after ordinary methods of fixation, or stain very slightly, and are not doubly refringent. This clear differentiation in the past has given one a feeling of security in distinguishing these two types of cells and the tumors which they compose, and with that idea in mind I am sure pathologists have made diagnoses in which they have felt a good deal of certainty, of pure theca cell and pure granulosa cell tumors. Of course, the fact that mixtures exist makes such diagnoses more difficult; however, if one limits oneself to differentiating the type of cell rather than the type of tumor, since it has been learned tonight that there are frequent mixtures, I should like to ask whether that apparently clearcut differentiation between the theca cell and the granulosa cell with respect to the types of lipid, paricularly the birefringence in the theca cell and its absence from the granulosa cell after luteinization of the granulosa cell, has been borne out by Dr. Traut's studies.

THOMAS A. BARRY: I should like to ask, first, whether these endometrial hyperplasias were all in the proliferative phase, and, second, how many endometrial carcinomas were associated with these tumors.

HERBERT F. TRAUT: In answering Dr. Liber, I have to disagree a little with him. In the first place, the differentiation between the normal theca cell and the normal granulosa cell has not been clearcut. Dr. Corner, for instance, who is probably the most prominent American student of the corpus luteum, found it extremely difficult to say which cells originated in the granulosa, and Schrader, in Germany, had similar difficulties, with the result, I think, that those who know most about the corpus luteum are in considerable doubt. I feel that that same doubt has been carried over into the neoplasms. I know that Loeffler and Priesel made clearcut diagnoses of neoplasms composed of theca cells, but I do not think the diagnosis in any instance was proved; Robert Meyer, for one, felt considerable doubt as to whether there was such a thing as a validly described theca cell tumor of the ovary, so I do not believe that when one applies the various polarized light criteria and ordinary staining reactions to neoplastic tissue one is quite sure to which cells one is applying them. Students of the matter still have a considerable way to go before they can clear up the question, but I believe they are starting in the right direction by studying the architecture of the more differentiated tumors, first taking out those which are essentially theca cells or essentially granulosa cells and studying their progression toward maturity. Pathologists make no claims to being able on all occasions to differentiate between these two types of cells. I doubt whether it can be done with great exactitude.

In regard to the hyperplasia—I mean the cystic glandular hyperplasia of the endometrium, which is always in a proliferative phase—we had no instance of carcinoma of the endometrium in connection with it.

Host-Parasite Relationships in Some Forms of Virus Encephalitis. LESTER S. KING (by invitation), Princeton, N. J.

In understanding host-parasite relationships it is desirable to know the complete life history of the infecting agent, including the possibility of its transmission by intermediate hosts and vectors, and the action of the agent on the host for which it is pathogenic. In the field of virus encephalitis the essential nature of the agents and their life histories are only imperfectly known, although in regard to a few diseases much information has recently been obtained concerning intermediate hosts and probable vectors and modes of entry into the body.

Knowledge is on firmer ground when a study is made of what happens after the virus is in the body. Three modes by which the virus enters the brain from the site of inoculation are clearly demonstrable: (1) It moves directly along nerve paths from the site of inoculation; (2) it is being transported by the blood to an indifferent area and passes to the brain by way of nerve paths from the new site; (3) it enters the brain directly from the blood stream. In reference to its passage along nerve paths, the differences between travel toward the cell body and travel away from the cell body are emphasized.

Many factors apart from immunologic considerations influence infection, applicable to some viruses although not to all. These include differences between host species and virus strains, as well as spontaneous and artificial variations in virus strains; the route of inoculation; the age of the animal; the presence of inflammation, and the recently observed effect of intraperitoneal glycerin.

The pathologic nature of certain virus diseases is considered. The primacy of inflammatory changes in the brain is stressed. Comparisons can be drawn between the action of the "Gordon agent" and the action of certain viruses, with special reference to neuronal necrosis and "inclusion bodies." Direct and indirect virus action must be distinguished.

DISCUSSION

MAURICE N. RICHTER: Do I understand correctly that you do not consider acidophil bodies in the nuclei as virus inclusions, Dr. King?

LESTER S. KING: No—I am sorry—I did not mean to give that impression. The intranuclear inclusions are divided into two types, the A and B inclusions, and I feel that type A, such as one finds in herpes, may well be specific inclusions, due to virus action. I think too much fuss has been made about type B inclusions, which are of the type described in equine encephalomyelitis, poliomyelitis and yellow fever encephalitis. These are small acidophilic bodies which investigators go to great effort to locate and which they feel have some important relationship to viruses. I do not see how that conclusion is reasonable. Every one knows that similar inclusion bodies are present in various tumors and other pathologic tissues. The inclusions which I illustrated here, caused by inoculation of an extract of bone marrow, are not caused by a virus. I feel that these type B inclusions are nonspecific and bear no necessary relation to the viruses.

MAURICE N. RICHTER, *President*

D. MURRAY ANGEVINE, *Secretary*

Regular Monthly Meeting, April 25, 1940

A Case of Torula Meningoencephalitis. LEO J. WADE (by invitation).

A 39 year old German-born housewife when admitted to the New York Hospital complained that she had suffered from headaches for four months and progressive loss of vision for two weeks. Physical examination disclosed papilledema

of 2 diopters, but repeated examination by several observers gave no constant or localizing neurologic signs. The white blood cell count was 5,200, with a normal differential count. The ventricular fluid was clear and contained 30 to 90 cells per cubic millimeter, more than 80 per cent of which were lymphocytes. Progressive increase in intracranial pressure necessitated repeated ventricular taps and finally a subtemporal decompression. Exploratory craniotomy failed to reveal any tumor. Torula histolytica was found in the ventricular fluid. The diagnosis of torulosis was confirmed by microscopic examination of a section of the meninges. The patient died on the third postoperative day, approximately five months after the onset of her illness.

At autopsy a thick white exudate was present over the frontal lobes, over the temporal poles and in the interpeduncular space. Torula histolytica was recovered in pure culture from this exudate. In the subarachnoid space about the lower two thirds of the cord was a grayish brown exudate. No gross lesions attributable to Torula were found in the parenchyma of the central nervous system.

A massive exudate consisting almost exclusively of mononuclear cells and foreign body giant cells was present in the subarachnoid space. The torulas were embedded in the exudate in a haphazard fashion, but they were especially numerous within the giant cells. No parenchymal lesion was found in the spinal cord, but numerous lesions were present in the temporal lobe. These were just beneath the cortex and consisted of cystlike spaces in which were clusters of the organisms, a few mononuclear cells and a delicate fibrin-like network between the organisms and cells.

DISCUSSION

MAURICE N. RICHTER: I should like to ask Dr. Wade if he will comment on the cultural characteristics of this organism. Several years ago Dr. Benham read a paper before this society on the cultural characteristics of a number of these yeastlike organisms, and she pointed out that the nomenclature was rather obscure. Many organisms have been classed under the same heading. Apparently the cultural characteristics would be important in deciding on the classification.

LEO J. WADE: My co-workers and I have studied Dr. Benham's paper and have repeated the bacteriologic examinations which she recommends for identifying the organisms. We found the organisms to behave in much the same manner as she reports. The organism grows very well in most mediums but perhaps best on Sabouraud's medium. The colonies form a yellow or cream-colored moist growth, which becomes darker with age. We inoculated various sugars and found that the organism forms acid but no gas with dextrose, levulose, galactose, sucrose and mannose, but it forms neither acid nor gas in any of the other commonly used sugars. Slight liquefaction of gelatin occurs in six to eight weeks. The microscopic appearance is characteristic. The organisms are rounded or slightly oval budding cells, the walls of which are highly refractive. This is perhaps best shown with fresh india ink preparations.

Human Tissue Reaction to an Unidentified Organism Resembling Actinobacillus Lignieresi and Pasteurella Pseudotuberculosis. T. J. CURPHEY.

In a recent report J. I. Schleifstein and M. B. Coleman described an unidentified organism resembling Actinobacillus lignieresi and Pasteurella pseudotuberculosis (*New York State J. Med.* **39**:1749, 1939). Five cultures of the identical strain of organism have been isolated during the last seventeen years. The present report deals with a fatal case of infection with this organism.

An 18 month old Jewish boy complained of fever eight days prior to entrance to the hospital. Two days after onset of the fever diarrhea developed, which increased in intensity until his admission to the hospital eight days after the onset of the condition. During the stay in the hospital the diarrhea increased and persisted until death occurred, with terminal hyperpyrexia, on the twelfth day of the

illness. The laboratory data were irrelevant; leukocytosis (20,200 white cells, 64 per cent of which were polymorphonuclear cells) was noted on the ninth day of the disease.

At autopsy the essential changes were in the gastrointestinal tract and the regional mesenteric lymph nodes. The small intestine from a point corresponding to the midjejunum to the terminal part of the ileum and the entire large intestine were the seat of diffuse discrete superficial areas of ulceration not larger than a pea. The regional lymph nodes were discretely enlarged and showed no noteworthy change on gross section. On microscopic examination of the ulcers, varying stages of the inflammatory process were evident, ranging from localized submucous inflammatory zones unattended by mucosal ulceration to shallow ulcers extending up to the muscular layer but not involving it. The involved area showed central actinomycotic-like granules, which with Gram's stain showed numerous gram-negative bacilli. Immediately surrounding these granules were numerous polymorphonuclear leukocytes enmeshed in an edematous reticulum. Peripheral to this zone, many mononucleated cells showing intracellular phagocytosed material were present. In an occasional area a zone of loose and recently proliferated fibroblasts could be demonstrated. Sections of the lymph nodes showed essentially the same change.

A culture of this unidentified organism was found to be pathogenic for guinea pigs and mice, producing histologic changes in the intestinal tract, liver and spleen similar to those noted in the human autopsy material. In addition, a Strauss reaction could be produced in the testicle of the guinea pig, which histologically showed the presence of the actinomycotic-like granules and the polymorphonuclear and mononuclear cell exudate.

The organism was a gram-negative bacillus showing a tendency to bipolar staining. It was not motile at 37 C. but showed motility at 20 to 22 C. It grew luxuriantly in broth, with the production of a heavy sediment. On Löffler's blood serum a creamy confluent growth was obtained, with no evidence of liquefaction of the medium. On a gelatin stab the organism grew readily on the surface but showed no liquefaction. Acid with traces of gas was produced in lactose, dextrose, saccharose, maltose, mannitol and dulcitol. It produced indol and caused a slightly acid change in litmus milk without the production of clot.

These bacteriologic features suggest a resemblance to Actinobacillus lignieresi and Pfeifferella whitmori (pseudomallei) and, to a lesser extent, to Pasteurella pseudotuberculosis. The bacteriologic evidence is as yet incomplete in respect to identification of this organism, but from the evidence submitted it appears not unlikely that the bacterium is a variant of the genus Actinobacillus. Before this can be established with certainty, however, further studies must be made.

A fatal case of intestinal ulceration of bacterial origin is reported. The identity of the organism is in doubt. On the one hand, if it is a variant of the genus Actinobacillus, the case is the second fatal case to be recorded in the literature. On the other hand, if it is not, the case is one of a new form of intestinal bacterial infection.

Postmortem Bacteriology. CASPAR G. BURN.

In view of the general belief that the data from postmortem bacteriologic examinations relate to bacterial invasion of the tissues after death, a systematic study was made of the bacterial flora of persons coming to autopsy. These subjects were unselected, included all ages and presented the usual disease processes observed in a general hospital. Emphasis was placed on the methods of culturing bacteria from material obtained at autopsies, for it is believed that the greatest difficulty is chiefly due to technical errors. The data reported are the summation of several independent observations of groups including from 127 to 200 autopsies in each. The results show that bacteria were obtained from the visceral organs with a significantly greater frequency than from the heart's blood. The order of

this frequency was: lungs, kidneys, liver, spleen and heart's blood. Bacillus coli, Staphylococci and Streptococcus viridans were isolated in greater frequency, while the more pathogenic strains, such as Pneumococcus, Streptococcus haemolyticus, Clostridium welchii, Haemophilus influenzae and others, were recovered but in a much lower incidence. The increase of bacteria in the organs was chiefly due to the three commonly isolated bacteria. The pathogens were usually found to occur with equal frequency in the organs and heart's blood.

The majority of the autopsies were made within three to eight hours after death. All bodies were placed at 10 C. within one half to three quarters of an hour after death. Statistical analysis revealed no significant differences in the bacterial content or in the kinds of bacteria found in the autopsy material studied thirteen to forty-eight hours after death as compared with that studied in the first four hours. However, the data do not include any information pertaining to invasion that might occur within the first hour after death. Studies in animals pertaining to postmortem invasion of bacteria showed that B. coli, Staphylococci and Cl. welchii were the organisms capable of growing and invading the tissues when the bodies were kept at room temperature. Pneumococci, Streptococcus nonhaemolyticus, Str. haemolyticus, H. influenzae, Pseudomonas pyocyanea and many others failed to invade even after ninety-six hours at room temperature. The ice box temperature (10 C.) prevented invasion of these strains, even after seven days. Higher temperature (37 C.) did not induce invasion by Ps. pyocyanea and some of the other noninvasive bacteria. Massive doses of B. coli were inoculated in the tissues immediately after death, but no evidence of invasion was observed several hours later.

Another method used in evaluating postmortem bacteriologic data was that of comparing postmortem blood cultures with those obtained during life. The two blood cultures were not alike in 87 (41 per cent) of the 212 cases, while in 125 (59 per cent) they were in complete agreement. Most of the differences in the 87 cases were due to the fact that one or the other sample gave a negative blood culture. A factor directly related to this discrepancy was the time between the last clinical culture and the death of the patient. Of this group, there were 28 cases (32 per cent) in which blood for the last clinical culture was taken within forty-eight hours before death, while over 80 per cent of the blood cultures in agreement were taken within this time. There were 5 blood cultures, 3 made post mortem and 2 clinically, in which the organisms were contaminants. Among the 65 instances in which both clinical and postmortem blood cultures were negative were 49 (75 per cent) in which pathogenic strains of bacteria and associated lesions were observed at autopsy. There is evidence to suggest that an unrecognized transient bacteremia occurred in many of these cases. Additional information regarding the 147 positive clinical and postmortem blood cultures showed that 41 (28 per cent) had more than one type of bacteria. Furthermore, these mixed cultures could be related to lesions at autopsy.

Postmortem invasion can occur as regards certain kinds of organisms but is prevented by proper treatment of the body after death. Therefore, bacteriologic examination of postmortem material is of value provided it is done under controlled conditions and not in a haphazard manner.

DISCUSSION

VERA DOLGOPOL: Can Dr. Burn explain the discrepancy between positive blood cultures some forty-eight hours before death and negative postmortem cultures? Are these perhaps influenced by chemotherapy—by drugs of the sulfanilamide group?

AMOUR F. LIBER: I should like to know if material for culture was taken from the paranasal sinuses or ear cavities. Material is often taken from those cavities at postmortem examination, and it is common experience that the positive culture obtained is just a contaminant from the nasal fossa and is not apparently correlated with infectious or toxic lesions elsewhere in the body.

CASPAR G. BURN: In regard to the question about sulfanilamide, most of this work was done before sulfanilamide came into use. I do not know what influence it would have on postmortem cultures. I presume it would sterilize the blood in many instances. My associates and I are beginning to study quite a number of cases in which sulfanilamide has been given, and we shall see what the result will be.

In regard to the paranasal sinuses, we did take a large number of cultures from sinuses and middle ears at autopsies, and we did find bacteria in apparently anatomically normal sinuses. We concluded that the presence of bacteria was by direct extension into the ear. We ruled out those cases in which we obtained positive cultures from the sinuses and also from the ears, unless there was pus or some lesion present. We took a number of cultures from the teeth at autopsy and found quite a number of the teeth had bacteria about the apexes. When the teeth were carious and the pulp exposed, we had difficulty in interpreting our results, as we were particularly interested in trying to find out whether localization from the blood stream occurred around the apexes of normal teeth. Of the normal teeth which we cultured in cases in which we had positive blood cultures, we found that about 10 per cent were infected with the organism, even though the organism had disappeared from the blood stream.

AMOUR F. LIBER: May I ask Dr. Burn to tell about the technic for obtaining cultures from the apexes of teeth?

CASPAR G. BURN: Dr. Burkett, who is a dentist and knows exactly where to obtain such cultures, did most of the work. We first took roentgenograms of the mouth routinely, of the upper and lower jaws. We were particularly interested in apical infection, so we took out the jaw that showed roentgen evidence of rarefaction around the apex, including also some normal teeth. The portion of the jaw which was removed was filled in by plasticene. The block of teeth was taken to the laboratory; the mucous membrane was removed, and iodine poured over the surface of the alveolar bone for sterilization; then the surface of bone was cultured to insure purity; we made an entrance into the apex with dental burrs, and a portion of the apical area of the tooth was removed; we also cultured the pulp in many cases. We found that 10 per cent of the normal teeth gave positive cultures from normal apical areas, and there was a much higher incidence of positive cultures from abnormal teeth.

Intracranial Pathways for the Extension of Infection from the Sphenoethmoid Sinuses. RUDOLPH KRAMER (by invitation) and MAX L. SOM (by invitation).

This paper emphasizes the pathologic and therapeutic importance of studying the pathways of infection from the ethmoid and sphenoid sinuses to the intracranial content. The method of taking sinus blocks was described, and a lantern slide demonstration of the following pathways of infection was given.

1. Osteitis and osteomyelitis.
2. Venous channels.
3. Perineural lymphatic spaces.
4. Perivascular lymphatic spaces.
5. Dehiscences.
6. Persistent cranial pharyngeal pouch.

DISCUSSION

SHELDON A. JACOBSON: It would not be easy to account for the lack of resistance on the part of this bone to the spread of infection except by taking into consideration the peculiar inertia of the bones of the skull, which has been

admirably shown again in the paper by Drs. Kramer and Som. Fractures of the skull occurring in children heal only very slowly, and those occurring in adults, sometimes not at all. An experimental study of this in the rat was made some time ago, in which the impression was gained that there is also a primary inertia of the calvarial periosteum, so that, unlike the other bones, the bones of the skull possess relatively low faculty to reproduce new bone when insulted. These slides provide confirmation of this. I scanned every one of these photomicrographs for evidence of reactive ossification, which anywhere else in the skeleton would be a striking evidence of the presence of inflammation, and only in one slide did I see a small area which might have been such a reactive ossification. It is a curious characteristic of the bones of the skull which I think is here reestablished.

Book Reviews

Shock: Blood Studies as a Guide to Therapy. John Scudder, M.D., Med. Sc.D., F.A.C.S., with a foreword by Allen O. Whipple, M.D. Cloth. Pp. XVII + 315, with 55 illustrations and 5 plates. Price $5.50. Philadelphia: J. B. Lippincott Company, 1940.

The book consists of 315 pages, 110 of which are occupied by figures, charts, diagrams and tabulations and 79 pages by bibliography and index. The use of space is not well proportioned to the importance of subject matter. The reader is provided with 21 blank pages for recording memoranda. Historical data and all preceding developments concerning shock are summarized in 8 pages. Traumatic toxemia is dealt with, pro and contra, in less than a page; 16 pages are devoted to potassium and 5 to other alterations in the blood. The historical treatment of shock is well reviewed in 20 pages. Hemoconcentration heralds the approach of shock hours before the blood pressure declines, and aids in detecting hemorrhage. Early treatment with adrenal cortical extract and salt solution and with transfusion of blood or of plasma was effective in many cases. The benefits were ascribed to the action of those agents "in combating the underlying vasoconstriction." It is stated that hemoconcentration precedes hyperpotassemia; hence it appears that the latter is not a primary etiologic factor, and its importance seems unduly emphasized. The author interprets it as indicating profound cellular injury but does not suggest the nature and origin of the injury. Capillary permeability is not mentioned, nor a possible relationship between endothelial damage and hemoconcentration. The latter is explained cryptically as "a measure of vasoconstriction." One section of 38 pages gives authors' names in chronologic order with a sentence or two epitomizing the contribution of each. The reader is expected to evaluate these from the fragments given, and to formulate his own conclusions.

The author does not correlate the results of previous investigations with his own work in support of one hypothesis or another. A new conception of shock is formulated on the basis of an altered chemical composition of the blood, with vasoconstriction as a fundamental characteristic and factor. The origin and mechanism of these are not explained, nor is evidence shown that they are causes rather than results. This conception, to be impressive, should be integrated closely with known facts regarding shock, with physiologic principles, with capillary reactions and endothelial function and with the visceral pathologic changes of shock. If the author understands these relationships he should depict them for the reader. One feels that the birth of this conception was not preceded by an adequate period of gestation.

Clinical Roentgenology of the Alimentary Tract. Jacob Buckstein, M.D., Visiting Roentgenologist (Alimentary Tract Division), Bellevue Hospital, New York; Consultant in Gastroenterology, Central Islip Hospital. Cloth. Pp. 652, with 525 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1940.

This book appropriately opens with a short chapter on the history of roentgenology and its application to the alimentary canal. Evidently the book has been written with attention to the needs of physicians who are not intimately acquainted with the value of the roentgen ray in the study of diseases of that tract. The book reflects well the importance of roentgen diagnosis in connection with the diseases of the esophagus, stomach, small intestine and colon. The significance of roentgenologic observations in those diseases and the evaluation of the criteria in interpretation are presented clearly and concisely. In addition to the chapters

on diseases of the alimentary tract proper, there are chapters on diseases of the gallbladder and diaphragm and on abnormalities of the liver, spleen and pancreas. The relations between the clinical aspects of the various diseases in question and the corresponding roentgen changes are illustrated in a large number of cases with, in many instances, descriptions of the gross appearances of the organs involved. This comparison of the roentgen appearances with the actual anatomic-pathologic conditions makes the book one of special interest to the pathologist. The chapter on chronic gastritis seems rather short, but the author emphasizes that this is due to the lack of results of adequate roentgen studies of this important process. There are more than 500 illustrations, mostly reproductions of roentgenograms, but also photographs of pathologic specimens. The literature has been well reviewed, and there is an extensive, completely indexed bibliography. The author's inclusion of several references under a single number may prove somewhat confusing. The salient features of the book are the close integration of the results of clinical, roentgen and pathologic examinations and the direct evaluation of the importance of roentgen examination in the study of gastrointestinal diseases.

Manuel de classification et de détermination des bactéries anaérobies. André R. Prévot, chef de service-adjoint a l'Institut Pasteur. Paper. Pp. 223. Paris: Masson & Cie, 1940.

The author states in the preface that he has had seventeen years of experience in working with anaerobes. He has been interested in the classification of anaerobes and is a member of the International Committee on the Classification of Bacteria. His purpose in this manual is to afford a working classification of these organisms. He was urged by his friends in the French army to get out rapidly a book which would be helpful to the laboratory staff in classifying anaerobes from various wound infections. This book was put out in response to that demand.

The author believes that the three most important features in classifying anaerobes are: spore formation, Gram staining and motility. He, of course, takes into account morphologic aspects, general physiology, cultural and biochemical reactions, pathogenicity and serologic and immunologic reactions. The book is comprehensive and catalogs the nonsporulating as well as the sporulating anaerobes, cocci, actinomycetes and spirochetes.

Many new families and genera have been added. For instance, Bacillus tetani or Clostridium tetani is listed in the order Plectridiales, the family Plectridiaceae and the genus Plectridium and is called Plectridium tetani. Bacillus welchii or Clostridium welchii is in the family Clostridiaceae and in the genus Welchia and is called Welchia perfringens.

The creation of new names for the anaerobes is going to add to the long list of names which some of them now have.

Since the anaerobes have been much neglected by medical bacteriologists, this book should prove stimulating to physicians as well as to laboratory workers.

Biochemistry of Disease. Meyer Bodansky, Ph.D., M.D., Director of the John Sealy Memorial Laboratory and Professor of Pathological Chemistry, University of Texas School of Medicine, and Oscar Bodansky, Ph.D., M.D., Lecturer in Biochemistry, Graduate Division, Brooklyn College. Pp. 637. Price $8. New York: The Macmillan Company, 1940.

This volume is a contribution of major importance to medical reference literature. The authors are authoritative biochemists and are also graduates in medicine. They have written their book expressly for the use of physicians and medical students and have arranged it with the problems and convenience of these workers in mind. They attempt to make the present knowledge of, and particularly the recent advances in, biochemistry readily available for application in the understanding, diagnosis and treatment of disease. For this purpose, the subject matter is arranged according to clinical entities rather than according to biochemical system, and an extensive subject index is appended.

As is to be expected, the great scope of the subject matter precludes the presentation of the minutiae of the biochemistry of any particular disease. References to original authors are made in the text, and a selected bibliography at the end of each chapter is included for those who wish to proceed to such information. However, the material which is actually presented adequately summarizes the essential biochemical information which can be usefully applied by those who are not specializing in a given field. There are numerous tables, graphs and diagrams which are helpful. This volume can be a much used addition to the library of any physician or medical student.

Pathological Histology. Robertson F. Ogilvie, M.D., F.R.C.P. (Edinburgh), Lecturer in Pathology, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist, Deaconess Hospital, Edinburgh; Examiner in Pathology for the Triple Qualification. Foreword by A. Murray Drennan, M.D., F.R.C.P. (Edinburgh), Professor of Pathology, University of Edinburgh. Cloth. Pp. 340, with 220 illustrations in color. Price $8.50. Baltimore: The Williams & Wilkins Company, 1940.

The purpose of this book is to facilitate the microscopic study of structural changes in disease. The text consists mainly of brief but clear descriptions of the gross and microscopic appearances of the more common morbid processes. The first six chapters deal with disturbances of nutrition, circulatory changes, inflammation and repair, specific inflammations and tumors. The remaining chapters cover the circulatory, respiratory, alimentary, urinary, reproductive, hemopoietic, nervous and osseous systems, and the endocrine glands. The distinctive feature of the book is the illustration of the text with 220 photographs in color of typical microscopic appearances. In each case the staining method and the magnification are given. "The pictures are reproduced from actual color-photographs [Finlay process] of the stained sections and both the original photograph and reproduction faithfully represent what may be observed with the microscope." The photographs are uniformly excellent in every respect and convey the most realistic impressions. The book will be found a valuable aid in the study of pathologic morphology.

A Textbook of Pathology. W. G. MacCallum, Professor of Pathology and Bacteriology, Johns Hopkins University, Baltimore. Seventh edition, thoroughly revised. Cloth. Pp. 1,302, with 697 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1940.

Attention is called to the review of the sixth edition in the ARCHIVES OF PATHOLOGY (**22**:865, 1936). The number of pages has grown from 1,277 to 1,302, but the number of illustrations remains the same. There are evidences of careful revision of the text as well as of the references at the end of the chapters. The book retains its place as a leading textbook of pathology.

La créatine: étude physio-pathologique. Jean Vague and Jean Dunan. Preface by Jean Roche. Paper. Pp. 256. Price 60 francs. Paris: Masson & Cie, 1939.

The authors have assembled the world's literature on all aspects of creatine metabolism. The treatment of the subject matter is systematic and covers the chemistry of creatine and its possible precursors, its metabolic functions, the mechanisms regulating its behavior in the body and its path of excretion. Little space is exclusively devoted to the clinical aspects of the subject. However, frequent references of clinical interest are made throughout the text.

As a text the book suffers from the attempt of the authors to deal with every paper on, or related to, the subject. The treatment is often sketchy and uncritical, especially in the sections dealing with hormonal and neuromuscular regulation. However, this volume will serve as a very useful reference work for any one particularly concerned with the biochemistry and metabolism of creatine and creatinine.

Books Received

CANCER. A MANUAL FOR PRACTITIONERS. The Committee on Publication (Representing the Massachusetts Medical Society and the American Society for the Control of Cancer): George W. Holmes, M.D., chairman; Channing C. Simmons, M.D., editor; Ernest M. Daland, M.D.; Shields Warren, M.D. Cloth. Pp. 284. Boston: Massachusetts Department of Public Health, Division of Adult Hygiene, 1940.

MIXOMATOSI. VIRUS MALATTIA DI SANARELLI. RICERCHE SPERIMENTALI. Prof. Gennaro di Macco and others. Preface by Prof. Giuseppe Sanarelli. Paper. Pp. 256, illustrated. Price 76 lire. Turin, Italy: Industrie Tipografico Editoriali Riunite, 1940.

NEOPLASTIC DISEASES. James Ewing, A.M., M.D., Sc.D., LL.D., professor of oncology at Cornell University Medical College; consulting pathologist, Memorial Hospital. Fourth edition, revised. Cloth. Pp. 1,160, with 581 illustrations. Price $14. Philadelphia and London: W. B. Saunders Company, 1940.

THE ENAMEL OF HUMAN TEETH: AN INQUIRY INTO THE FORMATION OF NORMAL AND HYPOPLASTIC ENAMEL MATRIX AND ITS CALCIFICATION. Moses Diamond and Joseph P. Weinman. Pp. 105, with 51 illustrations. Price $1.50. New York: Columbia University Press, 1940.

MANUEL DE CLASSIFICATION ET DE DÉTERMINATION DES BACTÉRIES ANAÉROBIES. André R. Prévot, chef de service-adjoint a l'Institut Pasteur, membre du Comité International de Nomenclature Bactérienne. Paper. Pp. 224, with 1 illustration. Price $1.75. Paris: Masson & Cie, 1940.

MÉDICAMENTS ANTIANÉMIQUES ET ANÉMIES EXPÉRIMENTALES. Jean Cheymol, D.Sc., pharmacien des hôpitaux de Paris, assistant à la Faculté de Médecine. Preface by Prof. René Hazard. Paper. Pp. 104. Price 22 francs. Paris: Masson & Cie, 1940.

PATHOGENIC ANAEROBIC ORGANISMS OF THE ACTINOMYCES GROUP. Dagny Erikson. Medical Research Council, Special Report Series, no. 240. Paper. Pp. 63, with 31 illustrations. Price 30 cents. New York: British Library of Information; London: His Majesty's Stationery Office, 1940.